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(54) **Use of 13C or 14C-labelled amino acids or peptides as dignostic agents for pancreatic exocrine function**

Verwendung von 13C oder 14C-markierten Aminosäuren oder Peptiden als Diagnosemittel für die exokrin Pankreasfunktion

Utilisation d' acides aminés ou de peptides marqués au 13C ou au 14C comme agents de diagnostique de la fonction exocrine pancréatique

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(56) References cited:

EP-A- 0 989 137	**EP-A- 1 089 765**
EP-A- 1 101 499	**WO-A-80/01415**
WO-A-98/33531	**US-A- 4 676 974**

- **YAMATO C. ET AL: "Hydrolysis and metabolism of N-benzoyl-L-tyrosyl-p-aminobenzoic acid in normal and pancreatic duct-ligated animals." JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, (1978) 206/2 (468-474). CODEN: JPETAB, XP001053537**
- **MORRIS ET AL: 'IN VITRO AND EX VIVO.....' BIOORGANIC MED. CHEM. vol. 5, no. 5, 1997, pages 797 - 808**
- **MITRA ET AL: '13C NMR study....' INT. J. PEPTIDE PROT. REOS. vol. 22, 1983, pages 502 - 508**
- **THANABAL ET AL: 'The 13C chemical shifts' J. OF BIOMOLECULAR NMR vol. 4, 1994, pages 47 - 59**
- **HELLER J. ET AL: 'Solid-state NMR studies.....' PROTEIN SCIENCE vol. 5, 1996, pages 1655 - 1661**
- **EVENEPOEL ET AL: 'EGG Protein assimilation in ....' 1996, LEUVEN - BELGIUM,**

Remarks:
The file contains technical information submitted after the application was filed and not included in this specification

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Description

BACKGROUND OF THE INVENTION

Field of the Invention:

[0001] The present invention relates to diagnostic agents for pancreatic exocrine function and novel compounds.

Background of the Invention:

[0002] "Pancreatic exocrine function tests" are useful for the diagnosis of pancreatic diseases such as chronic and acute pancreatitis and pancreatic cancer. It is also useful to assess the condition and the prognosis of patients and to control the medication of protease preparations: The general descriptions are found in Arvanitakis and Cooke, Gastroenterology, 74:932 (1978); Niederau and Grendell, Gastroenterology, 88:1973 (1985); Goldberg, Bull. Mol. Biol. Med., 15:1 (1990); Lankisch, Int. J. Pancreatology, 14:9 (1993); Bank and Chow, Gastroenterologist, 2:224 (1994); and Steer et al., Eng. J. Med., 332:1482 (1995).

[0003] The pancreatic exocrine function tests are roughly classified into intubation tests and tubeless tests. The intubation tests involve intubating a tube through the mouth to the duodenum to collect the duodenal juice. The secretin test is commonly used wherein secretin is intravenously administered to stimulate secretion of the pancreatic juice prior to the collection. This method is highly accurate since the amounts and components of the pancreatic juice are directly analyzed and is the "gold standard" of pancreatic exocrine function test. However, this method can not be used repeatedly or used for screening because of the very strong stress caused on the patients. It is not available at only a relatively small number of medical centers since the physician must be highly skilled. Further, since this method requires fluoroscopic tube placement during the collection of the duodenal juice, there is the problem of X ray exposure.

[0004] On the other hand, tubeless tests are easy to perform for estimating the pancreatic exocrine function which requires no intubation, wherein the excreted amount of compounds produced by pancreatic exocrine enzymes or the excreted amount of the pancreatic exocrine enzymes per se are measured. At present, the following four methods are mainly used:

1. PFD test wherein a synthetic substrate BT-PABA (N-benzoyl-L-tyrosyl-p-aminobenzoic acid) for chymotrypsin secreted from the pancreas is orally administered and the amount of PABA (p-aminobenzoic acid), a product degradated by chymotrypsin, excreted into the urine is measured;
Yamato C. et al. "Hydrolysis and Metabolism of N-benzoyl-L-tyrosyl-p-aminobenzoic acid in normal and pancreatic duct-ligated animals" Journal of Pharmacology and Experimental Therapeutics, (1978) 206/2 (468-474) reports a study on the hydrolysis and metabolism of N-benzoyl-L-tyrosyl-p-aminobenzoic acid (Bz-ty-PABA).
2. PLT test wherein a synthetic substrate FDL (fluorescein dilaurate) for cholesterol ester hydrolase, esterase, secreted from the pancreas is orally administered and the amount of the degradation product fluorescein excreted into the urine or the concentration thereof in the blood is measured;
3. Fecal chymotrypsin test wherein chymotrypsin in the feces is quantitatively determined; and
4. Fecal elastase test wherein elastase in the feces is quantitatively determined.

[0005] However, the sensitivity any of these tests is too low to detect slight decreases of pancreatic exocrine function. Therefore, they have not been used that often in recent years.

[0006] To solve this problem, many easier pancreatic exocrine function tests have been searched for; ^{13}C-breath tests have also been applied wherein a ^{13}C-labeled compound is administered and an increase of the concentration of $^{13}CO_2$ in the exhalation is measured. Examples of such ^{13}C-breath tests are illustrated below:

1. ^{13}C-breath test wherein a ^{13}C-labeled lipid or mixed triglyceride, which is a substrate for lipase, is administered: Chen et al., J. Nuclear Med., 15:1125 (1974); Watkins et al., J. Lab. Clin. Med., 90:422 (1977); Ghoos et al., Digestion, 22:239 (1981); John, SG., Gastroenterology, 83:44 (1982); Watkins et al., Gastroenterology, 82:911 (1982); Benini et al., Digestion, 29:91 (1984); Jones et al., J. Lab. Clin. Med., 105:647 (1985); Knoblach et al., Monatsschr Kinderheilkd, 136:26 (1988); Vantrappen et al., Gastroenterology, 96:1126 (1989); Murphy et al., Arch. Disease in Childhood, 65:574 (1990); Kato et al., Am. J. Gastroenterol., 88:64 (1993); McClean et al., Arch. Disease in Childhood, 69:366 (1993); Jakobs et al., Eur. J. Pediatr., 156: S78 (1997); and Kalivianakis et al., Eur. J. Clin. Invest., 27:434 (1997);
2. ^{13}C-breath test wherein a ^{13}C-labeled cholesterol ester, which is a substrate for cholesterol esterase, a lipase, is administered: Mundlos, et al., Pediatric Res., 22:257 (1987); Cole et al., Gastroenterology, 93:1372 (1987); and Mundlos et al., Gut, 31:1324 (1990);
US 4,676,974 (Hofmann et al.) teaches determination of pancreatic exocrine function by a breath test method wherein a radioactive labelled ester such as cholesterol octanoate is ingested in the body and is hydrolyzed by pancreatic enzymes and oxidised to labelled CO_2 which is expired in the breath.
3. ^{13}C-breath test wherein a ^{13}C-labeled starch, which is a substrate for an amylase, is adminis-

tered: Hiele et al., Gastroenterology, 96:503 (1989); Dewit et al., Pediatric Res., 32:45 (1992); and Z. Gastroenterol., 35:187 (1997); and

4. ^{13}C-breath test wherein a ^{13}C-enriched egg protein, which is a protein having a ^{13}C-concentration increased up to 1.4 atm % from the natural abundance of 1.1 atm % by feeding a chicken with ^{13}C-leucine, and which is a substrate for a protease, is administered: Y. Ghoos, $^{13}CO_2$-Breath Tests at the laboratory "Digestion-Absorption", University Hospital Gasthuisberg, Leuven, Belgium (1996).

[0007] However, all these methods are less sensitive than the conventional ones and time-consuming. Therefore, these methods have not been established in clinical fields.

[0008] Thus, it is desirable that a highly sensitive pancreatic exocrine function test be developed that imparts low stress on the subject and give accurate results soon.

SUMMARY OF THE INVENTION

[0009] Accordingly, an object of the present invention is to provide a diagnostic agent for pancreatic exocrine function which leads to a highly sensitive test of pancreatic exocrine function imparting low stress on the subject and providing accurate results soon.

[0010] It is another object of the present invention to provide a novel compound for the pancreatic exocrine function test.

[0011] The present inventors have found that pancreatic exocrine function can be estimated with high sensitivity by orally administering a ^{13}C-labeled peptide compound to a normal rat and a rat with chronic pancreatitis and measuring the $^{13}CO_2$ concentration in the exhaled CO_2 after administration. Thus, the present invention was completed.

[0012] In a first aspect the present invention provides use of an amino acid or a peptide containing at least one ^{13}C or ^{14}C atom, or a pharmaceutically acceptable salt thereof other than Bz-Tyr-^{13}C-PABA in the manufacture of a diagnostic agent for pancreatic exocrine function, wherein the diagnostic agent is used in a breath test.

[0013] In one preferred aspect the amino acid molecule contains a ^{13}C- or ^{14}C atom.

[0014] In one preferred aspect the amino acid or peptide has a modifying or protecting group and the modifying or protecting group contains a ^{13}C or ^{14}C atom.

[0015] In one preferred aspect the amino acid or peptide is represented by the following formula (I):

$$X_1\text{-}R_1\text{-}Y_1 \qquad \text{(I)}$$

wherein X_1 is a hydrogen atom or a protecting group, R_1 is a peptide of 2 to 50 amino acids, an amino acid or a single bond,

Y_1 is an amino acid optionally having a protecting group. Preferably at least one of the amino acids in R_1 and Y_1, or at least an ester group in Y_1 when the amino acid in Y_1 is protected with the ester group, is ^{13}C- or ^{14}C-labelled.

[0016] In one preferred aspect the amino acid or peptide, or pharmaceutically acceptable salt thereof is capable of being a substrate of a protease or proteases such that it is subsequently capable of being decarboxylated to generate $^{13}CO_2$ or $^{14}CO_2$. Preferably the protease or proteases are pancreatic exocrine proteases. Preferably the pancreatic exocrine protease or proteases are selected from the group consisting of chymotrypsin, trypsin, elastase, and carboxypeptidases.

[0017] In a second aspect the present invention provides a ^{13}C- or ^{14}C-labelled compound or salt thereof for use in diagnosis wherein the ^{13}C- or ^{14}C-labelled compound is represented by the following formula (II):

$$X_2\text{-}R_2\text{-}Y_2\text{-}Z_1 \qquad \text{(II)}$$

wherein X_2 is a hydrogen atom or a protecting group,
R_2 is a peptide of 2 to 5 amino acids, an amino acid or a single bond,
Y_2 is an amino acid,
Z_1 is an amino acid optionally having a protecting group, and at least one of the amino acids in R_2,Y_2 and Z_1, or at least one of the protecting groups in X_2 and Z_1 when the protecting groups contain a carbon atom, is ^{13}C or ^{14}C-labelled,
and wherein

1) in the case where X_2 is a protecting group and R_2 is a single bond,

    1-i) when Z_1 is D-Ala, Y_2 is an amino acid other than D-Ala,
    1-ii) when Z_1 is Ala-OMe, Y_2 is an amino acid other than Leu,
    1-iii) when Z_1 is Pro-OMe, Y_2 is an amino acid other than Gly,

2) in the case where X_2 is a protecting group and R_2 is an amino acid,

    2-i) when Z_1 is D-Ala, Y_2 is an amino acid other than Val,
    2-ii) when Z_1 is Ala, Y_2 is an amino acid other than Leu,
    2-iii) when Z_1 is Gly-OEt, Y_2 is an amino acid other than Pro,

3) in the case where X_2 is a protecting group, R_2 is a peptide of 2 amino acids and Z_1 is Gln optionally

having SEt, Y_2 is an amino acid other than Ala,

4) in the case where X_2 is a hydrogen atom and R_2 is a single bond,

4-i) when Z_1 is Pro,Y_2 is an amino acid other than Ala,
4-ii) when Z_1 is Leu, Y_2 is an amino acid other than Gly,Val,Leu and Tyr,
4-iii) when Z_1 is Phe, Y_2 is an amino acid other than Gly,
4-iv) when Z_1 is Gly-OEt, Y_2 is an amino acid other than Gly,
4-v) when Z_1 is Ala-OMe, Y_2 is an amino acid other than Leu,

5) in the case where X_2 is a hydrogen atom and R_2 is an amino acid,

5-i) when Z_1 is Leu, Y_2 is an amino acid other than Pro,
5-ii) when Z_1 is Phe, Y_2 is an amino acid other than Pro,
5-iii) when Z_1 is Gly, Y_2 is an amino acid other than Gly,
5-iv) when Z_1 is Pro, Y_2 is an amino acid other than Leu,
5-v) when Z_1 is Met, Y_2 is an amino acid other than Asp,
5-vi) when Z_1 is Asn-Bzl, Y_2 is an amino acid other than Leu,

6) in the case where X_2 is a hydrogen atom and R_2 is a peptide of 2 amino acids,

6-i) when Z_1 is Leu, Y_2 is an amino acid other than Leu,
6-ii) when Z_1 is Ser, Y_2 is an amino acid other than Asp, and

7) in the case where X_2 is a hydrogen atom and R_2 is a peptide of 3 amino acids,

7-i) when Z_1 is Leu, Y_2 is an amino acid other than Phe,
7-ii) when Z_1 is Met, Y_2 is an amino acid other than Phe;

provided that the following compounds are excluded. Ala-Pro, Gly-Leu,Gly-Phe, Val-Leu, Leu-Leu, Tyr-Leu, Ac-D-Ala-D-Ala, Gly-Gly-OEt, Leu-Ala-OMe, Ac-Gly-Pro-OMe, Boc-Leu-Ala-OMe, Gly-Pro-Leu, Gly-Pro-Phe, Ala-Gly-Gly, Gly-Leu-Pro, Phe-Asp-Met, Gly-Leu-Pro, Dansyl-Tyr-Val-D-Ala, Cbz-Gly-Leu-Ala, Thr-Leu-Asn-Bzl, Z-Pro-Pro-Gly-OEt, Leu-Leu-Leu-Leu, Lys-Arg-Asp-Ser, Ac-Leu-Ala-Ala -Gln (NMe_2), Ac-Leu-Ala-Ala-Gln (NMe_2)-SEt, Tyr-Gly-Gly-Phe-Leu and Tyr-Gly-Gly-Phe-Met, wherein Ac is acetyl, Et is ethyl, Me is methyl, Boc is t-butyloxycarbonyl, Dansyl is dansyl, Cbz is carbobenzyloxy, Bzl is benzoyl, Z is benzyloxycarbonyl, SEt is ethanethiol, and NMe_2 is dimethylamino.

[0018] In one preferred aspect of the ^{13}C- or ^{14}C-labelled compound or salt thereof for use in the second aspect of the present invention

1) in the case where X_2 is a protecting group selected from the group consisting of Dansyl, Cbz, Ac and Z, and R_2 is an amino acid or a peptide of 2 amino acids,

1-i) when Z_1 is D-Ala, Y_2 is an amino acid other than Val,
1-ii) when Z_1 is Ala, Y_2 is an amino acid other than Leu,
1-iii) when Z_1 is Gly-OEt, Y_2 is an amino acid other than Pro,
1-iv) when Z_1 is Gln(NMe_2) or Gln(NMe_2)-SEt, Y_2 is an amino acid other than Ala,

2) in the case where X_2 is Ac or Boc, and R_2 is a single bond,

2-i) when Z_1 is D-Ala, Y_2 is an amino acid other than D-Ala,
2-ii) when Z_1 is Pro-OMe, Y_2 is an amino acid other than Gly,
2-iii) when Z_1 is Ala-OMe, Y_2 is an amino acid other than Leu,

3) in the case where X_2 is a hydrogen atom and R_2 is an amino acid or a peptide of 2 or 3 amino acids,

3-i) when Z_1 is Leu, Y_2 is an amino acid other than Pro, Leu and Phe,
3-ii) when Z_1 is Phe, Y_2 is an amino acid other than Pro,
3-iii) when Z_1 is Gly, Y_2 is an amino acid other than Gly,
3-iv) when Z_1 is Pro, Y_2 is an amino acid other than Leu,
3-v) when Z_1 is Met, Y_2 is an amino acid other than Asp and Phe,
3-vi) when Z_1 is Asn-Bzl, Y_2 is an amino acid other than Leu,
3-vii) when Z_1 is Ser, Y_2 is an amino acid other than Asp, and

4) in the case where X_2 is a hydrogen atom and R_2 is a single bond,

4-i) when Z_1 is Pro, Y_2 is an amino acid other than Ala,
4-ii) when Z_1 is Leu , Y_2 is an amino acid other than Gly,Val,Leu and Tyr,
4-iii) when Z_1 is Phe, Y_2 is an amino acid other than Gly,
4-iv) when Z_1 is Gly-OEt, Y_2 is an amino acid

other than Gly,

4-v) when Z_1 is Ala-OMe, Y_2 is an amino acid other than Leu.

[0019] In one preferred aspect of the ^{13}C- or ^{14}C-labelled compound or salt thereof for use in the second aspect of the present invention X_2 is selected from the group consisting of Ac, Bz (benzoyl), Boc, Z and a hydrogen atom.

[0020] In one preferred aspect the ^{13}C- or ^{14}C-labelled compound or salt thereof for use in the second aspect of the present invention is capable of being a substrate of a protease or proteases such that it is subsequently capable of being decarboxylated to generate $^{13}CO_2$ or $^{14}CO_2$. Preferably the protease or proteases are pancreatic exocrine proteases. Preferably the pancreatic exocrine protease or proteases are selected from the group consisting of chymotrypsin, trypsin, elastase, and carboxypeptidases.

[0021] In one preferred aspect of the ^{13}C- or ^{14}C-labelled compound or salt thereof for use in the second aspect of the present invention X_2 is a protecting group and R_2 is a single bond.

[0022] In one preferred aspect of the ^{13}C- or ^{14}C-labelled compound or salt thereof for use in the second aspect of the present invention

(1) at least one of the amino acids in Y_2 and Z_1 is Arg or Lys,

(2) at least one of the amino acids in Y_2 and Z_1 is an aromatic amino acid, Leu, His or Met,

(3) at least one of the amino acids in Y_2 and Z_1 is a neutral and non-aromatic amino acid,

(4) the amino acid in Z_1 is an amino acid other than Arg, Lys and Pro, or

(5) the amino acid in Z_1 is Arg or Lys.

[0023] In one preferred aspect of the ^{13}C- or ^{14}C-labelled compound or salt thereof for use in the second aspect of the present invention X_2 is selected from the group consisting of a hydrogen atom, Bz, Ac and Boc, Y_2 is selected from the group consisting of Phe, Ala, Gly, Tyr and Arg, Z_1 is selected from the group consisting of Leu optionally having a protecting group, Ala optionally having a protecting group, and Gly optionally having a protecting group. Preferably Z_1 is selected from the group consisting of Leu, Ala, Gly, Leu-OMe, Leu-OEt, Ala-OMe, Ala-OEt, Gly-OMe and Gly-OEt.

[0024] In one preferred aspect of the ^{13}C- or ^{14}C-labelled compound or salt thereof for use in the second aspect of the present invention Z_1 is a ^{13}C- or ^{14}C-labelled amino acid optionally having a protecting group.

[0025] In one preferred aspect the ^{13}C- or ^{14}C-labelled compound or salt thereof for use in the second aspect of the present invention is selected from the group consisting of the following compounds:

(a) Phe-^{13}C-Leu,
(b) Arg-^{13}C-Leu,
(c) Bz-Ala-^{13}C-ALa,
(d) Bz-Gly-^{13}C-Leu,
(e) Bz-Phe-^{13}C-Gly,
(f) Bz-Tyr-^{13}C-Leu,
(g) Bz-Phe-^{13}C-Leu,
(h) Bz-(DL)Phe-^{13}C-Leu,
(j) Bz-Arg-^{13}C-Leu,
(k) Ac-Phe-^{13}C-Leu,
(l) Ac-Tyr-^{13}C-Leu,
(m) Bz-Ala-^{13}C-Ala-OMe,
(n) Bz-Gly-^{13}C-Leu-OMe,
(o) Bz-Phe-^{13}C-Gly-OMe,
(p) Bz-Phe-^{13}C-Leu-OMe,
(q) Bz-(DL)Phe-^{13}C-Leu-OMe,
(r) Ac-Phe-^{13}C-Leu-OMe,
(s) Ac-Tyr-^{13}C-Leu-OMe,
(t) Bz-Ala-Ala-Ala-Ala-Gly-Phe-^{13}C-Leu,
(u) Boc-Ala-Ala-Ala-Ala-Gly-Phe-^{13}C-Leu, and
(v) Bz-Ala-Ala-Ala-Ala-^{13}C-Gly-Phe-Leu

[0026] In a third aspect the present invention provides use of a ^{13}C- or ^{14}C-labelled compound as defined the second aspect of the invention or a pharmaceutically acceptable salt thereof in the manufacture of a diagnostic agent for pancreatic exocrine function, wherein the diagnostic agent is used in a breath test.

[0027] In a fourth aspect the present invention provides use of a ^{13}C- or ^{14}C-labelled compound as defined the second aspect of the invention or a pharmaceutically acceptable salt thereof in the manufacture of a diagnostic agent for chronic pancreatitis or acute pancreatitis.

BRIEF DESCRIPTION OF THE DRAWINGS

[0028]

Fig. 1 shows chymotrypsinogen and amylase contents in the pancreas of an oleic acid-injected group and a control group. For the oleic acid-injected group (•), 50 μl of oleic acid was injected into the pancreatic duct of 17 Wistar male rats of 5-weeks old (n=17). For the control group (O), only laparotomy was carried out (n=11). After the treatment, the rats of both groups were kept for 3 weeks. Then, the pancreas were removed and the chymotrypsinogen and amylase contents were determined.

Fig. 2 shows the time course of degree of increase of the ^{13}C concentration in the exhaled $^{13}CO_2$ ($\Delta^{13}C$(‰)) after administration of Bz-DL-Phe-(^{13}C-Leu)-Na. At 0 minute, Bz-DL-Phe-(^{13}C-Leu)-Na (250 mg/kg) was orally administered to the chronic pancreatitis rats (dotted line, n=8) and the control rats (solid line, n=4).

Fig. 3 shows the distribution of values for the chronic pancreatitis rats (•, n=8) and the normal rats (O,

n=4) in the PFD and Bz-DL-Phe-(^{13}C-Leu) (^{13}C-BPL) breath tests. Each rat was subjected to the PFD test prior to the Bz-DL-Phe-(^{13}C-Leu) breath test. The sensitivity (the ratio of true test positives to total true positives) is shown below for each distribution drawing when a cut off value (bar) is set such that the specificity (the ratio of true test negative to total true negatives) is 100%.

Fig. 4 shows the distribution of values for the chronic pancreatitis rats (•, n=8) and the normal rats (O, n=7) in the PFD and Bz-Ala-(^{13}C-Ala) (^{13}C-BAA) breath tests. Each rat was subjected to the PFD test prior to the Bz-Ala-(^{13}C-Ala) breath test. The sensitivity (the ratio of true test positives to total true positives) is shown below for each distribution drawing when a cut off value (bar) is set such that the specificity (the ratio of true test negative to total true negatives) is 100%.

Fig. 5 shows the distribution of values for the chronic pancreatitis rats (•, n=10) and the normal rats (O, n=10) in the PFD and Bz-Gly-(^{13}C-Leu) (^{13}C-BGL) breath tests. Each rat was subjected to the PFD test prior to the Bz-Gly-(^{13}C-Leu) breath test. The sensitivity (the ratio of true test positives to total true positives) is shown below for each distribution drawing when a cut off value (bar) is set such that the specificity (the ratio of true test negative to total true negatives) is 100%.

DESCRIPTION OF THE INVENTION

[0029] Hereinafter, the present invention will be described in detail.

[0030] Peptides are herein indicated in such a manner that the N-termini are on the left and the C-termini are on the right.

[0031] Amino acid residues are shown in three-letter abbreviations. Unless otherwise indicated, amino acids are L-isomers.

[0032] The "amino acid" refers herein to any compounds having carboxyl and amino groups in the molecule and includes imino acids such as proline and hydroxyproline and compounds having a lactam structure in the molecule.

[0033] The "peptide" refers herein to any compounds which are formed by linking at least two amino acids via a peptide bond and includes homomeric peptides consisting of amino acids, heteromeric peptides comprising a non-amino acid component(s), and their derivatives. The peptide has less than or equal to 100 amino acid residues.

[0034] The "amino acid or peptide containing at least one ^{13}C or ^{14}C atom" refers herein to any amino acid or peptide in which at least one carbon atom present in the amino acid, amino acid residues of the peptide, a modifying group or a protecting group thereof is replaced with a ^{13}C or ^{14}C atom, resulting in enriched ^{13}C or ^{14}C atoms in the amino acid or peptide molecules than found in nature.

[0035] The diagnostic agent for pancreatic exocrine function according to the present invention comprises an amino acid or a peptide containing at least one ^{13}C or ^{14}C atom, or a pharmaceutically acceptable salt thereof other than Bz-Tyr-^{13}C-PABA. The amino acid molecule may contain a ^{13}C or ^{14}C atom. Alternatively, when the amino acid or peptide has a modifying or protecting group, the modifying or protecting group may contain a ^{13}C or ^{14}C atom.

[0036] For example, the amino acid or peptide may be represented by the following formula (I):

$$X_1\text{-}R_1\text{-}Y_1 \qquad (I)$$

wherein X_1 is a hydrogen atom or a protecting group,
R_1 is a peptide of 2 to 50 amino acids, an amino acid or a single bond,
Y_1 is an amino acid optionally having a protecting group.

[0037] In the formula (I), X_1 is a hydrogen atom or a protecting group. The protecting group includes any protecting groups which are generally used in the field of organic chemistry, for example, those described in " Textbook for Biochemical Experiments 1 - Protein Chemistry IV", edited by Japan Biochemical Society, published by Tokyo Kagaku Dojin (1977); "Textbook for Experimental Chemistry 22 - Organic Synthesis IV", edited by Japan Chemical Society, published by Maruzen (1992); "Bases and Experiments of Peptide Synthesis", Nobuo Izumiya, Tetsuo Kato, Tohiko Aoyagi and Michinori Waki, published by Maruzen (1985); "Modification of Proteins", ed by Robert E. Feeney, John R. Whitaker, the American Chemical Society (1982); M. Bodanszky, "Principles of Peptides Synthesis", Springer-Verlag, Berlin (1984); and E. Schroder, K. Lubke, "The Peptides", Academic Press, N.Y. Vol. 1 (1965), Vol. 2 (1966). Concretely, examples thereof include benzoyl, acetyl, benzyloxycarbonyl, substituted benzyloxycarbonyl (such as p-nitrobenzyloxycarbonyl, p-methoxybenzyloxycarbonyl, etc.), t-butyloxycarbonyl, 9-fluorenylmethoxycarbonyl, p-toluenesulfonyl, phthalyl, formyl, trifluoroacetyl, triphenylmethyl, cyclohexyloxycarbonyl, o-nitrophenylsulfenyl, t-acyloxycarbonyl, isobornyloxycarbonyl, diphenylphosphinyl, diphenylphosphinothioyl, benzyl, alkyl, allylthiocarbonyl, o-nitrophenoxyacetyl, chloroacetyl, benzenesulfonyl, dibenzylphosphoryl, trialkylsilyl, allylidene, and acetoacetyl groups.

[0038] R_1 is a peptide of 2 to 50 amino acids, an amino acid or a single bond. The amino acid includes glycine, alanine, valine, leucine, isoleucine, serine, threonine, cystein, methionine, phenylalanine, tyrosine, tryptophan, aspartic acid, asparagine, glutamic acid, glutamine, arginine, lysine, histidine, proline, and ornithine.

[0039] Y_1 is an amino acid optionally having a protecting group. The protecting group includes any protecting

groups which are generally used in the field of organic chemistry, for example, those described in "Textbook for Biochemical Experiments 1 - Protein Chemistry IV", edited by Japan Biochemical Society, published by Tokyo Kagaku Dojin (1977); "Textbook for Experimental Chemistry 22 - Organic Synthesis IV", edited by Japan Chemical Society, published by Maruzen (1992); "Bases and Experiments of Peptide Synthesis", Nobuo Izumiya, Tetsuo Kato, Tohiko Aoyagi and Michinori Waki, published by Maruzen (1985); Modification of Proteins", ed by Robert E. Feeney, John R. Whitaker, the American Chemical Society (1982); M. Bodanszky, "Principles of Peptides Synthesis", Springer-Verlag, Berlin (1984); and E. Schroder, K. Lubke, "The Peptides", Academic Press, N.Y. Vol. 1 (1965), Vol. 2 (1966). Concretely, examples thereof include methyl ester, ethyl ester, benzyl ester, t-butyl ester and p-nitrobenzyl ester groups and N'-substituted hydrazide groups for protecting carboxyl groups; benzyloxycarbonyl, p-toluenesulfonyl and 2-chlorobenzyloxycarbonyl groups for protecting the ω-amino group in lysine and ornithine residues; nitro, methoxybenzyloxycarbonyl and p-toluenesulfonyl groups for protecting the guanidino group in arginine residue; benzyl and t-butyl groups for protecting the hydroxyl group in hydroxyl group-containing amino acid residues such as serine and tyrosine residues; benzyloxycarbonyl and benzyloxymethyl groups for protecting the imidazole group in histidine residue; benzyl and trityl groups for protecting the mercapto group in cystein residue; sulfoxide group for protecting the thioether group in methionine residue; and formyl group for protecting the indole group in tryptophan residue. The amino acid includes glycine, alanine, valine, leucine, isoleucine, serine, threonine, cystein, methionine, phenylalanine, tyrosine, tryptophan, aspartic acid, asparagine, glutamic acid, glutamine, arginine, lysine, histidine, proline, and ornithine.

[0040] The amino acids represented by R_1 and Y_1 and the peptides represented by R_1 may be modified in various manners. Such modification includes guanidylation, succinylation and acetylation of amino groups; modification of the guanidino group in arginine with a dicarbonyl compound; esterification of carboxyl groups; sulfenylsulfonation and alkylation of the thiol group in cystein; ethoxycarbonylation of the imidazole group in histidine; formation of sulfonium salts of methionine; acetylation of serine and threonine; nitration and iodination of tyrosine; and nitrophenylsulfonylation of tryptophan.

[0041] At least one of the amino acids in R_1, or at least an ester group in Y_1 when the amino acid in Y_1 is protected with the ester group, may be ^{13}C- or ^{14}C-labeled. Preferably, amino acid residues on which pancreatic exocrine proteases react are ^{13}C- or ^{14}C-labeled. The expression "^{13}C- or ^{14}C-labeled" used herein means that a molecule is marked by introducing thereinto ^{13}C or ^{14}C and includes substitution of a constitutive carbon of a molecule with ^{13}C or ^{14}C or covalent bonding of a molecule to a ^{13}C or ^{14}C-containing atomic group or molecule.

[0042] The present invention also encompasses a ^{13}C- or ^{14}C-labeled compound represented by the following formula (II):

$$X_2\text{-}R_2\text{-}Y_2\text{-}Z_1 \qquad \text{(II)}$$

or a salt thereof, for use in diagnosis
wherein X_2 is a hydrogen atom or a protecting group,
R_2 is a peptide of 2 to 5 amino acids, an amino acid or a single bond,
Y_2 is an amino acid,
Z_1 is an amino acid optionally having a protecting group, and
at least one of the amino acids in R_2, Y_2 and Z_1, or at least one of the protecting groups in X_2 and Z_1 when the protecting groups contain a carbon atom, is ^{13}C or ^{14}C-labeled,
provided that the following compounds are excluded. Ala-Pro, Gly-Leu,Gly-Phe, Val-Leu,Leu-Leu, Tyr-Leu, Ac-D-Ala-D-Ala, Gly-Gly-OEt, Leu-Ala-OMe, Ac-Gly-Pro-OMe, Boc-Leu-Ala-OMe, Gly-Pro-Leu, Gly-Pro-Phe, Ala-Gly-Gly, Gly-Leu-Pro, Phe-Asp-Met, Gly-Leu-Pro, Dansyl-Tyr-Val-D-Ala, Cbz-Gly-Leu-Ala, Thr-Leu-Asn-Bzl, Z-Pro-Pro-Gly-OEt, Leu-Leu-Leu-Leu, Lys-Arg-Asp-Ser, Ac-Leu-Ala-Ala-Gln(NMe_2), Ac-Leu-Ala-Ala-Gln(NMe_2)-SEt, Tyr-Gly-Gly-Phe-Leu and Tyr-Gly-Gly-Phe-Met, wherein Ac is acetyl, Et is ethyl, Me is methyl, Boc is t-butyloxycarbonyl, Cbz is carbobenzyloxy, Bzl is benzoyl, Z is benzyloxycarbonyl, SEt is ethanethiol, and NMe_2 is dimethylamino.

[0043] In the formula (II), X_2 is a hydrogen atom or a protecting group. The protecting group includes any protecting groups which are generally used in the field of organic chemistry, for example, those described in "Textbook for Biochemical Experiments 1 - Protein Chemistry IV", edited by Japan Biochemical Society, published by Tokyo Kagaku Dojin (1977); "Textbook for Experimental Chemistry 22 - Organic Synthesis IV", edited by Japan Chemical Society, published by Maruzen (1992); "Bases and Experiments of Peptide Synthesis", Nobuo Izumiya, Tetsuo Kato, Tohiko Aoyagi and Michinori Waki, published by Maruzen (1985); "Modification of Proteins", ed by Robert E. Feeney, John R. Whitaker, the American Chemical Society (1982); M. Bodanszky, "Principles of Peptides Synthesis", Springer-Verlag, Berlin (1984); and E. Schroder, K. Lubke, "The Peptides", Academic Press, N.Y. Vol. 1 (1965), Vol. 2 (1966). Concretely, examples thereof include benzoyl, acetyl, benzyloxycarbonyl, substituted benzyloxycarbonyl (such as p-nitrobenzyloxycarbonyl, p-methoxybenzyloxycarbonyl, etc.), t-butyloxycarbonyl, 9-fluorenylmethoxycarbonyl, p-toluenesulfonyl, phthalyl, formyl, trifluoroacetyl, triphenylmethyl, cyclohexyloxycarbonyl, o-nitrophenylsulfenyl, t-acyloxycarbonyl, isobornyloxycarb-

onyl, diphenylphosphinyl, diphenylphosphinothioyl, benzyl, alkyl, allylthiocarbonyl, o-nitrophenoxyacetyl, chloroacetyl, benzenesulfonyl, dibenzylphosphoryl, trialkylsilyl, allylidene, and acetoacetyl groups.

[0044] R_2 is a peptide of 2 to 5 amino acids, an amino acid or a single bond. The amino acid includes glycine, alanine, valine, leucine, isoleucine, serine, threonine, cystein, methionine, phenylalanine, tyrosine, tryptophan, aspartic acid, asparagine, glutamic acid, glutamine, arginine, lysine, histidine, proline, and ornithine.

[0045] Y_2 is an amino acid and the amino acid includes glycine, alanine, valine, leucine, isoleucine, serine, threonine, cystein, methionine, phenylalanine, tyrosine, tryptophan, aspartic acid, asparagine, glutamic acid, glutamine, arginine, lysine, histidine, proline, and ornithine.

[0046] Z_1 is an amino acid optionally having a protecting group: The protecting group includes any protecting groups which are generally used in the field of organic chemistry, for example, those described in "Textbook for Biochemical Experiments 1 - Protein Chemistry IV", edited by Japan Biochemical Society, published by Tokyo Kagaku Dojin (1977); "Textbook for Experimental Chemistry 22 - Organic Synthesis IV", edited by Japan Chemical Society, published by Maruzen (1992); "Bases and Experiments of Peptide Synthesis", Nobuo Izumiya, Tetsuo Kato, Tohiko Aoyagi and Michinori Waki, published by Maruzen (1985); "Modification of Proteins", ed by Robert E. Feeney, John R. Whitaker, the American Chemical Society (1982); M. Bodanszky, "Principles of Peptides Synthesis", Springer-Verlag, Berlin (1984); and E. Schroder, K. Lubke, "The Peptides", Academic Press, N.Y. Vol. 1 (1965), Vol. 2 (1966). Concretely, examples thereof include methyl ester, ethyl ester, benzyl ester, t-butyl ester and p-nitrobenzyl ester groups and N'-substituted hydrazide groups for protecting carboxyl groups; benzyloxycarbonyl, p-toluenesulfonyl and 2-chlorobenzyloxycarbonyl groups for protecting the ω-amino group
in lysine and ornithine residues; nitro, methoxybenzyloxycarbonyl and p-toluenesulfonyl groups for protecting the guanidino group in arginine residue; benzyl and t-butyl groups for protecting the hydroxyl group in hydroxyl group-containing amino acid residues such as serine and tyrosine residues; benzyloxycarbonyl and benzyloxymethyl groups for protecting the imidazole group in histidine residue; benzyl and trityl groups for protecting the mercapto group in cystein residue; sulfoxide group for protecting the thioether group in methionine residue; and formyl group for protecting the indole group in tryptophan residue. The amino acid includes glycine, alanine, valine, leucine, isoleucine, serine, threonine, cystein, methionine, phenylalanine, tyrosine, tryptophan, aspartic acid, asparagine, glutamic acid, glutamine, arginine, lysine, histidine, proline, and ornithine.

[0047] The amino acids represented by R_2, Y_2 and Z_1 and the peptides represented by R_2 may be modified in various manners. Such modification includes guanidylation, succinylation and acetylation of amino groups; modification of the guanidino group in arginine with a dicarbonyl compound; esterification of carboxyl groups; sulfenylsulfonation and alkylation of the thiol group in cystein; ethoxycarbonylation of the imidazole group in histidine; formation of sulfonium salts of methionine; acetylation of serine and threonine; nitration and iodination of tyrosine; and nitrophenylsulfonylation of tryptophan.

[0048] At least one of the amino acids in R_2, Y_2 and Z_1, or at least one of the protecting groups in X_2 and Z_1 when the protecting groups contain a carbon atom, is ^{13}C or ^{14}C-labeled.

[0049] Preferably, Z_1 is a ^{13}C- or ^{14}C-labeled amino acid optionally having a protecting group.

[0050] In one embodiment of the present invention, the ^{13}C- or ^{14}C-labeled compound, or salt thereof may be as follows:

1) in the case where X_2 is a protecting group and R_2 is a single bond,

 1-i) when Z_1 is D-Ala, Y_2 is an amino acid other than D-Ala,
 1-ii) when Z_1 is Ala-OMe, Y_2 is an amino acid other than Leu,
 1-iii) when Z_1 is Pro-OMe, Y_2 is an amino acid other than Gly,

2) in the case where X_2 is a protecting group and R_2 is an amino acid,

 2-i) when Z_1 is D-Ala, Y_2 is an amino acid other than Val,
 2-ii) when Z_1 is Ala, Y_2 is an amino acid other than Leu,
 2-iii) when Z_1 is Gly-OEt, Y_2 is an amino acid other than Pro,

3) in the case where X_2 is a protecting group, R_2 is a peptide of 2 amino acids and Z_1 is Gln optionally having SEt, Y_2 is an amino acid other than Ala,

4) in the case where X_2 is a hydrogen atom and R_2 is a single bond,

 4-i) when Z_1 is Pro,Y_2 is an amino acid other than Ala,
 4-ii) when Z_1 is Leu , Y_2 is an amino acid other than Gly,Val,Leu and Tyr,
 4-iii) when Z_1 is Phe, Y_2 is an amino acid other than Gly,
 4-iv) when Z_1 is Gly-OEt, Y_2 is an amino acid other than Gly,
 4-v) when Z_1 is Ala-OMe, Y_2 is an amino acid other than Leu,

5) in the case where X_2 is a hydrogen atom and R_2 is an amino acid,

5-i) when Z_1 is Leu, Y_2 is an amino acid other than Pro,
5-ii) when Z_1 is Phe, Y_2 is an amino acid other than Pro,
5-iii) when Z_1 is Gly, Y_2 is an amino acid other than Gly,
5-iv) when Z_1 is Pro, Y_2 is an amino acid other than Leu,
5-v) when Z_1 is Met, Y_2 is an amino acid other than Asp,
5-vi) when Z_1 is Asn-Bzl, Y_2 is an amino acid other than Leu,

6) in the case where X_2 is a hydrogen atom and R_2 is a peptide of 2 mino acids,

6-i) when Z_1 is Leu, Y_2 is an amino acid other than Leu,
6-ii) when Z_1 is Ser, Y_2 is an amino acid other than Asp, and

7) in the case where X_2 is a hydrogen atom and R_2 is a peptide of 3 amino acids,

7-i) when Z_1 is Leu, Y_2 is an amino acid other than Phe,
7-ii) when Z_1 is Met, Y_2 is an amino acid other than Phe.

[0051] In another embodiment of the present invention, the ^{13}Cor ^{14}C-labeled compound, or salt thereof may be as follows:

1) in the case where X_2 is a protecting group selected from the group consisting of Dansyl, Cbz, Ac and Z, and R_2 is an amino acid or a peptide of 2 amino acids,

1-i) when Z_1 is D-Ala, Y_2 is an amino acid other than Val,
1-ii) when Z_1 is Ala, Y_2 is an amino acid other than Leu,
1-iii) when Z_1 is Gly-OEt, Y_2 is an amino acid other than Pro,
1-iv) when Z_1 is Gln(NMe_2) or Gln(NMe_2)-SEt, Y_2 is an amino acid other than Ala,

2) in the case where X_2 is Ac or Boc, and R_2 is a single bond,

2-i) when Z_1 is D-Ala, Y_2 is an amino acid other than D-Ala,
2-ii) when Z_1 is Pro-OMe, Y_2 is an amino acid other than Gly,
2-iii) when Z_1 is Ala-OMe, Y_2 is an amino acid other than Leu,

3) in the case where X_2 is a hydrogen atom and R_2 is an amino acid or a peptide of 2 or 3 amino acids,

3-i) when Z_1 is Leu, Y_2 is an amino acid other than Pro, Leu and Phe,
3-ii) when Z_1 is Phe, Y_2 is an amino acid other than Pro,
3-iii) when Z_1 is Gly, Y_2 is an amino acid other than Gly,
3-iv) when Z_1 is Pro, Y_2 is an amino acid other than Leu,
3-v) when Z_1 is Met, Y_2 is an amino acid other than Asp and Phe,
3-vi) when Z_1 is Asn-Bzl, Y_2 is an amino acid other than Leu,
3-vii) when Z_1 is Ser, Y_2 is an amino acid other than Asp, and

4) in the case where X_2 is a hydrogen atom and R_2 is a single bond,

4-i) when Z_1 is Pro,Y_2 is an amino acid other than Ala,
4-ii) when Z_1 is Leu , Y_2 is an amino acid other than Gly,Val,Leu and Tyr,
4-iii) when Z_1 is Phe, Y_2 is an amino acid other than Gly,
4-iv) when Z_1 is Gly-OEt, Y_2 is an amino acid other than Gly,
4-v) when Z_1 is Ala-OMe, Y_2 is an amino acid other than Leu.

[0052] In formula (II), it is preferred that X_2 is selected from the group consisting of Ac, Bz (benzoyl), Boc, Z and a hydrogen atom.

[0053] Also, in formula (II), it is preferred that X_2 is a protecting group and R_2 is a single bond.

[0054] In a preferred embodiment of the present invention,

(1) at least one of the amino acids in Y_2 and Z_1 is Arg or Lys (this is a suitable substrate for trypsin),
(2) at least one of the amino acids in Y_2 and Z_1 is an aromatic amino acid, Leu, His or Met (this is a suitable substrate for chymotrypsin),
(3) at least one of the amino acids in Y_2 and Z_1 is a neutral and non-aromatic amino acid (this is a suitable substrate for elastase),
(4) the amino acid in Z_1 is an amino acid other than Arg, Lys and Pro (this is a suitable substrate for carboxypeptidase A), or
(5) the amino acid in Z_1 is Arg or Lys (this is a suitable substrate for carboxypeptidase B).

[0055] In a more preferred embodiment of the present invention, X_2 is selected from the group consisting of a

hydrogen atom, Bz, Ac and Boc, Y_2 is selected from the group consisting of Phe, Ala, Gly, Tyr and Arg, Z_1 is selected from the group consisting of Leu optionally having a protecting group, Ala optionally having a protecting group, and Gly optionally having a protecting group, for example, the group consisting of Leu, Ala, Gly, Leu-OMe, Leu-OEt, Ala-OMe, Ala-OEt, Gly-OMe and Gly-OEt, and more specifically, the group consisting of ^{13}C- or ^{14}C-Leu, ^{13}C- or ^{14}C -Ala, ^{13}C- or ^{14}C-Gly, ^{13}C- or ^{14}C-Leu-OMe, ^{13}C- or ^{14}C-Leu-OEt, ^{13}C- or ^{14}C-Ala-OMe, ^{13}C- or ^{14}C-Ala-OEt, ^{13}C- or ^{14}C-Gly-OMe and ^{13}C- or ^{14}C-Gly-OEt.

[0056] In a still more preferred embodiment of the present invention, the ^{13}C- or ^{14}C-labeled compound represented by formula (II), or salt thereof is selected from the group consisting of the following compounds:

(a) Phe-^{13}C-Leu,
(b) Arg-^{13}C-Leu,
(c) Bz-Ala-^{13}C-ALa,
(d) Bz-Gly-^{13}C-Leu,
(e) Bz-Phe-^{13}C-Gly,
(f) Bz-Tyr-^{13}C-Leu,
(g) Bz-Phe-^{13}C-Leu,
(h) Bz-(DL)Phe-^{13}C-Leu,
(j) Bz-Arg-^{13}C-Leu,
(k) Ac-Phe-^{13}C-Leu,
(l) Ac-Tyr-^{13}C-Leu,
(m) Bz-Ala-^{13}C-Ala-OMe,
(n) Bz-Gly-^{13}C-Leu-OMe,
(o) Bz-Phe-^{13}C-Gly-OMe,
(p) Bz-Phe-^{13}C-Leu-OMe,
(q) Bz-(DL)Phe-^{13}C-Leu-OMe,
(r) Ac-Phe-^{13}C-Leu-OMe,
(s) Ac-Tyr-^{13}C-Leu-OMe,
(t) Bz-Ala-Ala-Ala-Ala-Gly-Phe-^{13}C-Leu,
(u) Boc-Ala-Ala-Ala-Ala-Gly-Phe-^{13}C-Leu, and
(v) Bz-Ala-Ala-Ala-Ala-^{13}C-Gly-Phe-Leu

[0057] The ^{13}C- or ^{14}C-labeled compounds represented by the above formula (I) and pharmaceutically acceptable salts thereof and the ^{13}C- or ^{14}C-labeled compounds represented by the above formula (II) and salts thereof may be absorbed through the digestive tract after the reaction of a protease or proteases, and decarboxylated by metabolic action to generate $^{13}CO_2$ or $^{14}CO_2$. The protease or proteases may be pancreatic exocrine proteases including chymotrypsin, trypsin, elastase, and carboxypeptidases represented by carboxypeptidase A and B.

[0058] Chymotrypsin specifically cleaves the carboxyl terminal peptide linkage of tyrosine, tryptophan, phenylalanine, leucine, histidine or methionine residue and also acts on esters and amides containing these residues.

[0059] Trypsin catalyzes the hydrolysis of peptide linkages at the carboxyl terminus of an arginine residue, a lysine residue, and an S-aminoethylcysteine residue produced by artificial reactions. Further, chemical synthetic products containing an (allyl)amide or ester linkage instead of the peptide linkage may be substrates for trypsin so long as they are derived from the three amino acids.

[0060] Elastase hydrolyzes the peptide linkage at the carboxyl terminus of alanine, glycine, valine, leucine, isoleucine and methionine residues, which are uncharged non-aromatic amino acids. It is known that succinyl-(alanyl)3-p-nitroanilide may be an artificial substrate therefor.

[0061] Carboxypeptidase B has an action to sequentially cleave from the carboxyl terminus basic amino acids such as arginine and lysine.

[0062] Carboxypeptidase A has an action to sequentially cleave from the carboxyl terminus aromatic hydrophobic amino acids such as tyrosine, phenylalanine, tryptophan, leucine, isoleucine, threonine, glutamine, histidine, alanine, valine, asparagine, serine, lysine, glycine, aspartic acid and glutamic acid.

[0063] By taking into consideration such substrate specificities of proteases as above described, ^{13}C- or ^{14}C-labeled compounds or salts thereof suitable for use in diagnostic agents for pancreatic exocrine function may be designed.

[0064] The ^{13}C- or ^{14}C-labeled compounds may be synthesized in a known manner using commercially available amino acids. For example, methods described in Textbook for Experimental Chemistry 22 - Organic Synthesis IV", edited by Japan Chemical Society, published by Maruzen (1992) may be used. One illustrative example thereof will be described below.

[0065] A ^{13}C-labeled amino acid is dissolved in hydrogen chloride/methanol and refluxed. The resulting methyl ester is suspended in dichloromethane and triethylamine is then dropwise added while being ice-cooled and stirred. Further, an N-benzoyl-amino acid, 1-hydroxy-1H-benzotriazole.H_20 (HOBt) and dichloromethane are added. Then, a solution of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide-HCl (WSC) dissolved in dichloromethane is added and the mixture is stirred. After concentration, the reaction mixture is extracted with ethyl acetate, washed with 1N HCl, 5% $NaHCO_3$, and water, dried over magnesium sulfate, and evaporated to dryness, or further saponified, to yield the desired ^{13}C-labeled compound represented by the formula (I).

[0066] The ^{13}C- or ^{14}C-labeled compounds may be obtained in the form of a salt. The salts may include those with inorganic acids such as hydrochloric, sulfuric, nitric and phosphoric acids; with organic acids such as formic, acetic, propionic, glycolic, succinic, malic, tartaric, citric and trifluoroacetic acids; with alkali metals such as sodium and potassium; with alkaline earth metals such as calcium; and with organic amines such as ammonium, ethanolamine, triethylamine and dicyclohexylamine.

[0067] The test using the agents for pancreatic exocrine function according to the present invention may be carried out by administering to a subject the ^{13}C- or ^{14}C-

labeled compound represented by the formula (I) or pharmaceutically acceptable salt thereof. A test is possible in which the concentration of the ^{13}C- or ^{14}C-labeled compound is measured in serum, urine or stool after the administration, however, a breath test is desirable in which an increase in ^{13}C or ^{14}C concentration is measured in the exhaled CO_2 after the administration. When the ^{13}C- or ^{14}C-labeled compound represented by the formula (I) or pharmaceutically acceptable salt thereof is administered to a subject, a test meal or the like may be ingested by the subject to induce secretion of pancreatic enzymes. Also, two or more ^{13}C- or ^{14}C-labeled compounds represented by the formula (I) or pharmaceutically acceptable salts thereof may be combined for use. Concretely, in the cases of ^{13}C, the ^{13}C concentration is determined in the exhaled CO_2 after the administration, then the pancreatic exocrine function is diagnosed from either the data of the degree of increase ($\Delta^{13}C$(‰)) of the ^{13}C concentration in the exhaled $^{13}CO_2$ at predetermined times (e.g., 5, 10 and 15 minutes) after the administration, or the data associated with the time course (onset slope, change in slope, peak time, etc.) in the degree of increase ($\Delta^{13}C$(‰)) of the ^{13}C concentration in the exhaled $^{13}CO_2$ during a predetermined period after the administration. In the cases of ^{14}C, the ^{14}C concentration, i.e., radioactivity, is determined in the exhaled CO_2 after the administration; and the pancreatic exocrine function is diagnosed from either the data of the quantity of radioactivity in the exhaled $^{13}CO_2$ at predetermined times (e.g., 5, 10 and 15 minutes) after the administration, or the data associated with the time course (onset slope, change in slope, peak time, etc.) in the rate increase of radioactivity in the exhaled $^{13}CO_2$ during a predetermined period after the administration. These test methods utilize the phenomenon that when the ^{13}C- or ^{14}C-labeled compound represented by the formula (I) or pharmaceutically acceptable salt thereof is administered to a subject, the compound is absorbed through the digestive tract after the reaction of a protease or proteases, and decarboxylated by metabolic action in the body to generate $^{13}CO_2$ or $^{14}CO_2$.

[0068] The ^{13}C concentration in the exhaled $^{13}CO_2$ can be determined by gas chromatography-mass spectrometry (GC-MS), infrared spectroscopy, mass spectrometry, photoelectric acoustic spectroscopy, NMR (nuclear magnetic resonance), and other methods.

[0069] The ^{14}C concentration or radioactivity in the exhaled CO_2 may be measured from the breath of a subject, directly or after trapping CO_2 in a solvent, with a GM counter, a liquid scintillation counter, a solid scintillation counter, autoradiography, an ionization chamber, or the like.

[0070] The diagnostic agent for pancreatic exocrine function according to the present invention may be formulated from the ^{13}C- or ^{14}C-labeled compound represented by the formula (I) or pharmaceutically acceptable salt thereof alone or in combination with an excipient or carrier into an oral preparation such as a tablet, capsule, powder, granule, liquid, etc. The excipient or carrier may be any pharmaceutically acceptable one ordinarily used in this field and its nature and composition may be appropriately chosen. For example, water may be used as a liquid carrier. Solid carriers include cellulose derivatives such as hydroxypropyl cellulose, and organic acid salts such as magnesium stearate. Also, freeze-dried preparations may be used.

[0071] The ^{13}C- or ^{14}C-labeled compound represented by the formula (I) or pharmaceutically acceptable salt thereof is contained in the agent in variable amounts depending on the nature of the agent, but generally in an amount of 1 to 100% by weight, preferably 50 to 100% by weight. In a capsule, tablet, granule or powder preparation, the ^{13}C- or ^{14}C-labeled compound represented by the formula (I) or pharmaceutically acceptable salt thereof is contained in the preparation in an amount of about 10 to 100% by weight, preferably 50 to 100% by weight, the balance being a carrier.

[0072] The dose of the diagnostic agent for pancreatic exocrine function according to the present invention should be sufficient to determine or confirm an increase of $^{13}CO_2$ or $^{14}CO_2$ in the breath after the administration. It will be varied depending upon the age and body weight of a subject and the purpose of the test. For example, the unit dose may be about 1 to 1000 mg/kg of body weight for an adult.

EXAMPLES

[0073] Hereinbelow, the present invention is illustrated in more detail by the following examples.

Example 1: Preparation of Bz-DL-Phe-(^{13}C-Leu)-OMe

[0074] After 1 g of 1-^{13}C-L-leucine (Masstrace) was dissolved in hydrogen chloride/methanol and refluxed, the resulting ^{13}C-L-leucine methyl ester was suspended in 50 ml of dichloromethane and 1.08 ml of triethylamine was added dropwise under while being ice-cooled and stirred. Further, 2.0 g of N-benzoyl-DL- phenylalanine, 2.34 g of HOBt (1-hydroxy-1H-benzotriazole·H_2O) and 50 ml of dichloromethane were added. Then, a solution of 1.49 g of WSC (1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide-HCl) dissolved in 100 ml of dichloromethane was added and stirred for 1 hour under while being ice-cooled and then overnight at room temperature. The completion of the reaction was confirmed by silica gel thin layer chromatography using chloroform:methanol (95:5) as a developing solvent. The reaction mixture was concentrated, extracted with ethyl acetate, washed with 1N-HCl, 5% $NaHCO_3$, and water, dried over magnesium sulfate, and concentrated to dryness to yield 2.32 g of Bz-DL-Phe-(^{13}C-Leu)-OMe.

Example 2: Preparation of Bz-DL-Phe-(^{13}C-Leu) and its sodium salt (Bz-DL-Phe- (^{13}C-Leu)·Na)

[0075] After 2.32 g of Bz-DL-Phe-(^{13}C-Leu)-OMe was dissolved in 100 ml of methanol, 6.4 ml of 1N NaOH was added dropwise under while being ice-cooled and stirred followed by heating and stirring at 70°C for 2.5 hours. The completion of the reaction was confirmed by silica gel thin layer chromatography using chloroform: methanol (95:5) as a developing solvent. After the reaction was completed, the reaction mixture was neutralized with 1N-HCl, concentrated and dissolved in 5% $NaHCO_3$-After washing with ethyl acetate, 5% $NaHCO_3$ was acidified with 1N-HCl. The reaction mixture was extracted with ethyl acetate, washed with water, dried over magnesium sulfate, and concentrated to dryness to yield 1.93 g of Bz-DL-Phe-(^{13}C-Leu), which was then recrystallized with ethyl acetate.

[0076] The structure and ^{13}C-labeled position were confirmed by ^{13}C-NMR and mass spectrometry.

^{13}C-NMR (methanol-d4, 300 MHz): 175.8 ppm (^{13}COOH)

Mass spectrometry (m/z): 383 (M^+), 365, 224, 131, 105, 77

LC-MS (m/z): 384 (M^++H), 252, 224, 105

[0077] The sodium salt of Bz-DL-Phe-(^{13}C-Leu) was obtained by neutralizing Bz-DL-Phe- (^{13}C-Leu) with an equivalent of 1M sodium carbonate followed by lyophilization.

Example 3: Degradation of Bz-DL-Phe-(^{13}C-Leu) by chymotrypsin

[0078] Bz-DL-Phe-(^{13}C-Leu) was reacted with chymotrypsin and the degradation product, leucine, generated upon action of chymotrypsin was quantitatively determined by ninhydrin reaction. The reaction was carried out in 20 mM HEPPS-Na (pH 8.0), 23 mM Bz-DL-Phe-(^{13}C-Leu), 0.16 mg/ml chymotrypsin (from bovine pancreas, Worthington Biochemical Corporation, #1432, Lot 37A906) at 37°C for 15 minutes. After reaction, 50 µl of citrate buffer (citric acid monohydrate), 20 µl of ninhydrin solution (50 mg/ml solution in methyl cellosolve) and 100 µl of KCN solution (0.01 M aqueous KCN solution diluted 50 times with methyl cellosolve) were added to 100 µl of the reaction mixture and heated at 100°C for 15 minutes. After cooling the ninhydrin reaction mixture to room temperature, 150 µl of 60% (V/V) ethanol was added to 100 µl of the ninhydrin reaction mixture and stirred followed by determination of an absorbance at a wave length of 570 nm. An experiment wherein all the reactions that were carried out without adding Bz-DL-Phe-(^{13}C-Leu) was taken as a "blank," and leucine was used as a standard.

[0079] Bz-DL-Phe-(^{13}C-Leu) was degraded upon reaction with chymotrypsin to produce leucine. The rate of degradation was 1.72 nmole/mg chymotrypsin/min. From these results, it was confirmed that Bz-DL-Phe-(^{13}C-Leu) could be a substrate for chymotrypsin.

Example 4: Preparation of Bz-Ala-(^{13}C-Ala)-OMe

[0080] After 2 g of 1-^{13}C-L-alanine (Masstrace) was dissolved in hydrogen chloride/methanol and refluxed, the resulting ^{13}C-L-alanine methyl ester was suspended in 100 ml of dichloromethane and 4.0 ml of triethylamine was added dropwise while being ice-cooled and stirred. Further, 5.6 g of N-benzoyl-L-alanine, 8.9 g of HOBt and 100 ml of dichloromethane were added. Then, a solution of 5.6 g of WSC dissolved in 200 ml of dichloromethane was added and stirred for 1 hour while being ice-cooled and then overnight at room temperature. The completion of the reaction was confirmed by silica gel thin layer chromatography using chloroform:methanol (95:5) as a developing solvent. The reaction mixture was concentrated, extracted with ethyl acetate, washed with 1N-HCl, 5% $NaHCO_3$, and water, dried over magnesium sulfate, and concentrated to dryness to yield 4.38 g of Bz-Ala-(^{13}C-Ala)-OMe.

Example 5: Preparation of Bz-Ala-(^{13}C-Ala) and its sodium salt

[0081] After 4.38 g of Bz-Ala-(^{13}C-Ala)-OMe was dissolved in 100 ml of methanol, 16 ml of 1N NaOH was added dropwise while being ice-cooled and stirred followed by heating and stirring at 70°C for 2 hours. The completion of the reaction was confirmed by silica gel thin layer chromatography using chloroform:methanol (95:5) as a developing solvent. After the reaction was completed, the reaction mixture was neutralized with 1N-HCl, concentrated and dissolved in 5% $NaHCO_3$. After washing with ethyl acetate, 5% $NaHCO_3$ was acidified with 1N-HCl. The reaction mixture was extracted with ethyl acetate, washed with water, dried over magnesium sulfate, and concentrated to dryness to yield 2.85 g of Bz-Ala-(^{13}C-Ala), which was then recrystallized with ethyl acetate.

[0082] The structure and ^{13}C-labeled position were confirmed by ^{13}C-NMR and mass spectrometry.

^{13}C-NMR (methanol-d4, 300 MHz): 175.9 ppm (^{13}COOH)

LC-MS (m/z): 266 (M^++H), 176, 148, 105

[0083] The sodium salt of Bz-Ala-(^{13}C-Ala) was obtained by neutralizing Bz-Ala-(^{13}C-Ala) with an equivalent of 1M sodium carbonate followed by lyophilization.

Example 6: Preparation of Bz-Gly-(^{13}C-Leu)-OMe

[0084] After 2 g of 1-^{13}C-L-leucine (Masstrace) was dissolved in hydrogen chloride/methanol and refluxed, the resulting ^{13}C-L-leucine methyl ester was suspended in 100 ml of dichloromethane and 2.4 ml of triethylamine was added dropwise while being ice-cooled and stirred. Further, 3.0 g of N-benzoylglycine, 5.1 g of HOBt and 100 ml of dichloromethane were added. Then, a solution

of 3.3 g of WSC dissolved in 200 ml of dichloromethane was added and stirred for 1 hour while being ice-cooled and then overnight at room temperature. The completion of the reaction was confirmed by silica gel thin layer chromatography using chloroform:methanol (95:5) as a developing solvent. The reaction mixture was concentrated, extracted with ethyl acetate, washed with 1N-HCl, 5% $NaHCO_3$, and water, dried over magnesium sulfate, and concentrated to dryness to yield 4.38 g of Bz-Gly-(^{13}C-Leu)-OMe.

Example 7: Preparation of Bz-Gly-(^{13}C-Leu) and its sodium salt

[0085] After 4.38 g of Bz-Gly-(^{13}C-Leu)-OMe was dissolved in 100 ml of methanol, 15 ml of 1N NaOH was added dropwise while being ice-cooled and stirred followed by heating and stirring at 70°C for 2.5 hours. The completion of the reaction was confirmed by silica gel thin layer chromatography using chloroform:methanol (95:5) as a developing solvent. After the reaction was completed, the reaction mixture was neutralized with 1N-HCl, concentrated and dissolved in 5% $NaHCO_3$. After washing with ethyl acetate, 5% $NaHCO_3$ was acidified with 1N-HCl. The reaction mixture was extracted with ethyl acetate, washed with water, dried over magnesium sulfate, and concentrated to dryness to yield 3.83 g of Bz-Gly-(^{13}C-Leu), which was then recrystallized with ethyl acetate.

[0086] The structure and ^{13}C-labeled position were confirmed by ^{13}C-NMR and mass spectrometry.
^{13}C-NMR ($CDCl_3$, 300 MHz): 175.5 ppm (^{13}COOH)
Mass spectrometry (m/z): 293 (M^+), 275, 134, 105, 77
LC-MS (m/z): 294 (M^-+H), 162, 134, 105

[0087] The sodium salt of Bz-Gly-(^{13}C-Leu) was obtained by neutralizing Bz-Gly-(^{13}C-Leu) with an equivalent of 1M sodium carbonate followed by lyophilization.

Example 8: Preparation of Bz-DL-Phe-(^{13}C-Gly)-OMe

[0088] After 570 mg of 1-^{13}C-glycine (Masstrace) was dissolved in hydrogen chloride/methanol and refluxed, the resulting ^{13}C-glycine methyl ester was suspended in 50 ml of dichloromethane and 1 ml of triethylamine was added dropwise while being ice-cooled and stirred. Further, 2.0 g of N-benzoyl-DL-phenylalanine, 2.3 g of HOBt and 50 ml of dichloromethane were added. Then, a solution of 1.41 g of WSC dissolved in 100 ml of dichloromethane was added and stirred for 1 hour while being ice-cooled and then overnight at room temperature. The completion of the reaction was confirmed by silica gel thin layer chromatography using chloroform:methanol (95:5) as a developing solvent. The reaction mixture was concentrated, extracted with ethyl acetate, washed with 1N-HCl, 5% $NaHCO_3$, and water, dried over magnesium sulfate, and concentrated to dryness to yield 2.11 g of Bz-DL-Phe-(^{13}C-Gly)-OMe.

Example 9: Preparation of Bz-DL-Phe-(^{13}C-Gly) and its sodium salt

[0089] After 2.11 g of Bz-DL-Phe-(^{13}C-Gly)-OMe was dissolved in 100 ml of methanol, 6.9 ml of 1N NaOH was added dropwise while being ice-cooled and stirred followed by heating and stirring at 70°C for 2.5 hours. The completion of the reaction was confirmed by silica gel thin layer chromatography using chloroform:methanol (95:5) as a developing solvent. After the reaction was completed, the reaction mixture was neutralized with 1N-HCl, concentrated and dissolved in 5% $NaHCO_3$. After washing with ethyl acetate, 5% $NaHCO_3$ was acidified with 1N-HCl. The reaction mixture was extracted with ethyl acetate, washed with water, dried over magnesium sulfate, and concentrated to dryness to yield 1.3 g of Bz-DL-Phe-(^{13}C-Gly).

[0090] The structure and ^{13}C-labeled position were confirmed by ^{13}C-NMR and mass spectrometry.
^{13}C-NMR ($CDCl_3$, 300 MHz) : 179.2 ppm (^{13}COOH)
Mass spectrometry (m/z): 327 (M^+), 309, 224, 161, 105, 77
LC-MS (m/z): 328 (M^++H), 252, 224, 105

[0091] The sodium salt of Bz-DL-Phe-(^{13}C-Gly) was obtained by neutralizing Bz-DL-Phe-(^{13}C-Gly) with an equivalent of 1M sodium carbonate followed by lyophilization.

Example 10: Preparation of Ac-Phe-(^{13}C-Leu)-OMe

[0092] After 1.16 g of 1-^{13}C-L-leucine (Masstrace) was dissolved in hydrogen chloride/methanol and refluxed, the resulting ^{13}C-L-leucine methyl ester was suspended in 50 ml of dichloromethane and 1.2 ml of triethylamine was added dropwise while being ice-cooled and stirred. Further, 1.8 g of N-acetyl- L-phenylalanine, 2.7 g of HOBt and 50 ml of dichloromethane were added. Then, a solution of 1.7 g of WSC dissolved in 100 ml of dichloromethane was added and stirred for 1 hour while being ice-cooled and then overnight at room temperature. The completion of the reaction was confirmed by silica gel thin layer chromatography using chloroform:methanol (95:5) as a developing solvent. The reaction mixture was concentrated, extracted with ethyl acetate, washed with 1N-HCl, 5% $NaHCO_3$, and water, dried over magnesium sulfate, and concentrated to dryness to yield 2.62 g of Ac-Phe-(^{13}C-Leu)-OMe.

Example 11: Preparation of Ac-Phe-(^{13}C-Leu) and its sodium salt

[0093] After 2.62 g of Ac-Phe-(^{13}C-Leu)-OMe was dissolved in 100 ml of methanol, 9.3 ml of 1N NaOH was added dropwise while being ice-cooled and stirred followed by heating and stirring at 70°C for 2.5 hours. The completion of the reaction was confirmed by silica gel thin layer chromatography using chloroform:methanol (95:5) as a developing solvent. After the reaction was

completed, the reaction mixture was neutralized with 1N-HCl, concentrated and dissolved in 5% $NaHCO_3$. After washing with ethyl acetate, 5% $NaHCO_3$ was acidified with 1N-HCl. The reaction mixture was extracted with ethyl acetate, washed with water, dried over magnesium sulfate, and concentrated to dryness to yield 2.44 g of Ac-Phe-(^{13}C-Leu), which was then recrystallized with ethyl acetate.

[0094] The structure and ^{13}C-labeled position were confirmed by ^{13}C-NMR and mass spectrometry.

^{13}C-NMR ($CDCl_3$, 300 MHz): 175.9 ppm (^{13}COOH)

LC-MS (m/z): 322 (M^{+}+H), 190, 162, 120

[0095] The sodium salt of Ac-Phe-(^{13}C-Leu) was obtained by neutralizing Ac-Phe-(^{13}C-Leu) with an equivalent of 1M sodium carbonate followed by lyophilization.

Example 12: Preparation of Ac-Tyr-(^{13}C-Leu)-OMe

[0096] After 0.98 g of 1-^{13}C-L-leucine (Masstrace) was dissolved in hydrogen chloride/methanol and refluxed, the resulting ^{13}C-L-leucine methyl ester was suspended in 50 ml of dichloromethane and 1.0 ml of triethylamine was added dropwise while being ice-cooled and stirred. Further, 1.65 g of N-acetyl-L-tyrosine, 2.26 g of HOBt and 50 ml of dichloromethane were added. Then, a solution of 1.42 g of WSC dissolved in 100 ml of dichloromethane was added and stirred for 1 hour while being ice-cooled and then overnight at room temperature. The completion of the reaction was confirmed by silica gel thin layer chromatography using chloroform:methanol (95:5) as a developing solvent. The reaction mixture was concentrated, extracted with ethyl acetate, washed with 1N-HCl, 5% $NaHCO_3$, and water, dried over magnesium sulfate, and concentrated to dryness to yield 2.14 g of Ac-Tyr-(^{13}C-Leu)-OMe.

Example 13: Preparation of Ac-Tyr-(^{13}C-Leu) and its sodium salt

[0097] After 2.14 g of Ac-Tyr-(^{13}C-Leu)-OMe was dissolved in 100 ml of methanol, 7.3 ml of 1N NaOH was added dropwise while being ice-cooled and stirred followed by heating and stirring at 70°C for 2.5 hours. The completion of the reaction was confirmed by silica gel thin layer chromatography using chloroform:methanol (95:5) as a developing solvent. After the reaction was completed, the reaction mixture was neutralized with 1N-HCl, concentrated and dissolved in 5% $NaHCO_3$. After washing with ethyl acetate, 5% $NaHCO_3$ was acidified with 1N-HCl. The reaction mixture was extracted with ethyl acetate, washed with water, dried over magnesium sulfate, and concentrated to dryness to yield 1.36 g of Ac-Tyr-(^{13}C-Leu), which was then recrystallized with ethyl acetate.

[0098] The structure and ^{13}C-labeled position were confirmed by ^{13}C-NMR and mass spectrometry.

^{13}C-NMR (methanol-d4, 300 MHz): 175.8 ppm (^{13}COOH)

LC-MS (m/z): 338 (M^{+}+H), 206, 178, 136

[0099] The sodium salt of Ac-Tyr-(^{13}C-Leu) was obtained by neutralizing Ac-Tyr-(^{13}C-Leu) with an equivalent of 1M sodium carbonate followed by lyophilization.

Example 14: Preparation of a model of chronic pancreatitis in rats 14-1 Method for preparation

[0100] A model of chronic pancreatitis in rats were prepared by injecting oleic acid into the pancreatic duct (Mundlos et al., Pancreas 1:29 (1986)). After overnight fast, a Wistar male rat of 5 weeks old was anesthetized by intraperitoneal administration of Nembutal (50 mg/kg). The abdominal wall was shaved and the rat was fixed supinely on an operating table. An Isodine solution was applied to sterilize, and a 3 to 4 cm midline incision was made on the abdomen. The duodenum and the pancreas were drawn out and a 25G needle was pierced through the duodenal wall. A polyethylene cannula (PE-10) was inserted through the pierced hole into the duodenum and further inserted into the common bile duct by about 5 mm from the papilla. The inserted cannula was fixed by a microclip. Further, the biliary duct was closed by a microclip in order to prevent oleic acid from flowing into the liver. Oleic acid (50 µl) was injected at a rate of 20 µl/min by a microsyringe pump. After the injection was completed, the rat was allowed to stand for 2 minutes so that the oleic acid was distributed throughout the pancreas. The microclips and cannula were removed and the duodenum was returned. The endothelium was sutured with silk thread (Nescosuture silk suture thread 3-0, Nihon Shoji KK) and the outer skin was sutured with a skin stapler (Appose ULC, No. 8034-12, 5.7 x 3.8 mm). As a control, only laparotomy was carried out. These rats subjected to the operation were kept under free intake of standard food and water at 23°C, relative humidity of 55% until use.

14-2 Evaluation

[0101] After injecting oleic acid into the pancreatic duct and being kept for 3 weeks, these rats (oleic acid-injected rats) were subjected to measurement of amylase in the blood, and to quantitative determination of chymotrypsinogen and amylase contents in the pancreas (Fig. 1). An extraction buffer (20 mM HEPPS+Na, pH 8.0, 100 mM KCl, 0.5% (w/v) Triton X-100) was added to the removed pancreas to the total volume of 10 ml. The material was subjected to ultrasonic disruption (Bionic 7250, Seiko, Sonics & Materials) and centrifuged at 10,000 x g for 20 minutes to yield a pancreatic extract as a supernatant. To 200 µl of the pancreatic extract, 200 µl 1 of 1 mg/ml trypsin (from porcine pancreas, Biozyme, code TRY1, batch 0196), which was 5 mg/ml trypsin, 1 mM acetate buffer, pH 3.2, five times diluted with 20 mM Hepes-Na, pH 8.0, was added and allowed to stand at 4°C for 2 hours to activate chymotrypsinogen to chymotrypsin (Lampel and Kern, Virchows Archiv A

373:97 (1977)). After adding 30 μl of the activated pancreatic extract and 20 μl of 123.8 mM BT-PABA to 150 μl of 20 mM HEPPS-Na, pH 8.0, the chymotrypsin reaction was performed at 37°C for 15 minutes. After the reaction, 10 μl of 100% (W/V) TCA solution was added and centrifuged at 15,000 x g for 5 minutes and the PABA in the supernatant was quantitatively determined using a PABA measuring kit (Eisai). The amylase activity in the pancreatic extract was measured using Fuji Dri-Chem. Both units of activity (U) show μ mole release/min.

[0102] The chymotrypsinogen content was 62.4 U ± 26.2, n=11 in the control rats and 8.1 U ± 10.7, n=17 in the oleic acid-injected rats. The amylase content was 8681 U ± 5622, n=11 in the control rats and 789 U ± 1842, n=17 in the oleic acid-injected rats. Thus, Both enzyme contents were significantly reduced in the oleic acid-injected rats (Fig. 1). In particular, the chymotrypsinogen content was markedly reduced. Therefore, pancreatitis can be evaluated to have occurred in 12 rats among 17 oleic acid-injected rats (70%), if the normal lower limit is the mean - 2SD of chymotrypsinogen content for the control. On the other hand, the blood amylase concentrations of both rats were at the same level, which increases in the acute period of pancreatitis, 1930 U ± 823, n=11 in the control rats and 2137 U ± 668, n=17 in the oleic acid-injected rats; therefore, the oleic acid-injected rats are characterized as a model of chronic pancreatitis.

Example 15: Bz-DL-Phe-(^{13}C-Leu) breath test

15-1 Method

[0103] The rats of the model of chronic pancreatitis and control rats, which were kept for 3 to 4 weeks after the operation, were made to fast from 9:00 AM. At 4:00 PM, 3 mg/ml of BT-PABA (PFD solution for internal use, Eisai) was orally administered in an amount of 15 mg/kg. Urine was collected for 16 hours until 9:00 AM of the next day. The amount of the collected urine and the PABA concentration in the urine were determined using a PABA measuring kit to determine the excretion rate in urine (PFD test).

[0104] After the PFD test, Bz-DL-Phe-(^{13}C-Leu)-Na dissolved in distilled water (250 mg/kg, 6 ml/kg) was orally administered to the rats, which fasted for 24 hours, to carry out a ^{13}C-breath test. The rat of 9 weeks old was fixed without anesthesia in a rat holder for a microwave irradiation apparatus. The breath was collected at a rate of about 100 to 300 ml/min using a stroke pump (Variable Stroke Pump VS-500, Shibata Kagaku Kogyo) and introduced directly to a flow cell of a $^{13}CO_2$ analyzer EX-130S (Nihon Bunko). A Perma Pure drier (MD-050-12P, Perma Pure INC.) was set between the rat holder and stroke pump to remove out water vapor in the breath. CO_2 concentration was stabilized, the rat was once removed out of the rat holder and Bz-DL-Phe-(^{13}C-Leu)-Na dissolved in distilled water was administered into the stomach using an oral sonde.

[0105] Output data from the $^{13}CO_2$ analyzer were AD converted and put into a personal computer (Apple Power Macintosh 8500) . Using a data processing soft ware Lab VIEW (National Instruments), 10 data points at every 100 msec were integrated and averaged in an interval of 5 seconds and converted to ^{13}C atom %, Δ^{13}C (‰), and CO_2 concentration (%). In this manner, the ^{13}C breath test was continuously carried out. The converted data were displayed in real time and stored in a hard disc. CO_2 concentration in the collected breath was held at 3 ± 0.5%.

[0106] Δ^{13}C (‰) was calculated from the ^{13}C concentration in the exhaled CO_2 at each time point (^{13}C tmin) and the ^{13}C concentration in standard CO_2 (^{13}C std) according to the following equation:

$$\Delta^{13}\mathrm{C}\ (‰) = [(^{13}\mathrm{C\ tmin} - {}^{13}\mathrm{C\ 0min})/{}^{13}\mathrm{C\ std}] \times 1000$$

[0107] After the breath test, the abdomen of the rat was cut opened under anesthesia by intraperitoneal administration of Nembutal (50 mg/kg) and the whole pancreas was removed and weighed. Then, the chymotrypsinogen content was determined.

15-2 Results

[0108] Bz-DL-Phe-(^{13}C-Leu) breath test was carried out (Fig. 2) wherein 250 mg/kg of Bz-DL-Phe-(^{13}C-Leu) -Na was orally administered to the chronic pancreatitis and control rats and the time course of the $^{13}CO_2$ concentration in the exhaled CO_2 after the administration was measured. In the control rats, the Δ^{13}C (‰) value began to increase at 2 to 3 minutes after the administration, although there was some difference in the degree of increase among individuals. The value reached a peak of 100 to 200 ‰ at 15 to 20 minutes and then gradually decreased. In 7 cases of the chronic pancreatitis rats, on the contrary, the degree of increase was small and continued to slowly increase for 30 minutes. The remaining one rat showed the same behavior as the control rats but the peak time was later. At 10 minutes after the administration, the Δ^{13}C (‰) values of the chronic pancreatitis rats were smaller than the smallest value of Δ^{13}C (‰) for the control rats. Accordingly, the sensitivity becomes 100% even when the cut off value is set such that the specificity is made to be 100% by using the Δ^{13}C (‰) value at 10 minutes as a check value (Fig. 3). On the other hand, the sensitivity in the PFD test of the same group of rats carried out immediately before the breath test was 50%, indicating that the Bz-DL-Phe-(^{13}C-Leu) breath test was far superior thereto (Fig. 3). Since it has been reported that the sensivity of simple tests for pancreatic exocrine function other than the PFD test is identical with that of the PFD test, the Bz-DL-Phe-(^{13}C-Leu) breath test can be said to be

the most highly sensitive simple test for pancreatic exocrine function. Further, in addition to the patient's stress because of 6 hours of collecting the urine and forced drinking of a large amount of water, this PFD test is disadvantaged in that subsequent analyses is necessary so that the results are often not found in the same day. On the contrary, the Bz-DL-Phe-(^{13}C-Leu) breath test has an advantage in that the restraint period is only 10 minutes and that the results can be known soon at that site and time.

Example 16: Bz-Ala-(^{13}C-Ala) breath test

[0109] In a similar manner to 15-1, Bz-Ala-(^{13}C-Ala) breath test was carried out wherein 50 mg/kg of Bz-Ala-(^{13}C-Ala)·Na was orally administered and the time course of the $^{13}CO_2$ concentration in the exhaled CO_2 after the administration was measured. The sensitivity was 88% when the Δ^{13}C (‰) value at 10 minutes was used as a check value and a cut off value was set such that the specificity was 100% (Fig. 4). On the other hand, the sensitivity in the PFD test of the same group of rats carried out immediately before the breath test was 63%; thus, the Bz-Ala-(^{13}C-Ala) breath test was higher in sensitivity (Fig. 4). Further, in addition to the patient's stress due to 6 hours of collecting the urine and forced drinking of a large amount of water, this PFD test is disadvantaged in that subsequent analyses is necessary so that the results are often not found in the same day. On the contrary, the Bz-Ala-(^{13}C-Ala) breath test could be said to be more excellent in that the restraint period is only 10 minutes and that the results can be known soon at that site and time.

Example 17: Bz-Gly-(^{13}C-Leu) breath test

[0110] In a similar manner to 15-1, Bz-Gly-(^{13}C-Leu) breath test was carried out wherein 50 mg/kg of Bz-Gly-(^{13}C-Leu)-Na was orally administered and the time course of the $^{13}CO_2$ concentration in the exhaled CO_2 after the administration was measured. The sensitivity was 80% when the Δ^{13}C (‰) value at 18 minutes was used as a check value and a cut off value was set such that the specificity was 100% (Fig. 5). On the other hand, the sensitivity in the PFD test of the same group of rats carried out immediately before the breath test was 50%; thus, the Bz-Gly-(^{13}C-Leu) breath test was higher in sensitivity (Fig. 5). Further, in addition to the patient's stress due to 6 hours of collecting the urine and forced drinking of a large amount of water, this PFD test is disadvantaged in that subsequent analyses is necessary so that the results are often not found in the same day. On the contrary, the Bz-Gly-(^{13}C-Leu) breath test has an advantage in that the restraint period is only 18 minutes and that the results can be known soon at that site and time.

Example 18: Preparation of Bz-L-Phe-(^{13}C-Leu)-OMe

[0111] After 3.02 g of 1-^{13}C-L-leucine (Masstrace) was dissolved in hydrogen chloride/methanol and refluxed, the resulting ^{13}C-L-leucine methyl ester was suspended in 150 ml of dichloromethane and 3.22 ml of triethylamine was added dropwise while being ice-cooled and stirred. Further, 6.16 g of N-benzoyl- L-phenylalanine, 7.02 g of HOBt and 100 ml of dichloromethane were added. Then, a solution of 4.4 g of WSC dissolved in 200 ml of dichloromethane was added and stirred for 1 hour while being ice-cooled and then overnight at room temperature. The completion of the reaction was confirmed by silica gel thin layer chromatography using chloroform:methanol (95:5) as a developing solvent. The reaction mixture was concentrated, extracted with ethyl acetate, washed with 1N-HCl, 5% $NaHCO_3$, and water, dried over magnesium sulfate, and concentrated to dryness to yield 8.36 g of Bz-L-Phe-(^{13}C-Leu)-OMe.

Example 19: Preparation of Bz-L-Phe-(^{13}C-Leu) and its sodium salt

[0112] After 8.36 g of Bz-L-Phe-(^{13}C-Leu)-OMe was dissolved in 150 ml of methanol, 23.2 ml of 1N NaOH was added dropwise while being ice-cooled and stirred followed by heating and stirring at 70°C for 3.5 hours. The completion of the reaction was confirmed by silica gel thin layer chromatography using chloroform:methanol (95:5) as a developing solvent. After the reaction was completed, the reaction mixture was neutralized with 1N-HCl, concentrated and dissolved in 5% $NaHCO_3$. After washing with ethyl acetate, 5% $NaHCO_3$ was acidified with 1N-HCl. The reaction mixture was extracted with ethyl acetate, washed with water, dried over magnesium sulfate, and concentrated to dryness to yield 7.92 g of Bz-L-Phe-(^{13}C-Leu), which was then recrystallized with ethyl acetate.

[0113] The structure and ^{13}C-labeled position were confirmed by ^{13}C-NMR and mass spectrometry.

^{13}C-NMR (methanol-d4, 300 MHz): 175.9 ppm (^{13}COOH)

Mass spectrometry (m/z): 383 (M^+), 365, 224, 131, 105, 77

LC-MS (m/z): 384 (M^++H), 252, 224, 105

[0114] The sodium salt of Bz-L-Phe-(^{13}C-Leu) was obtained by neutralizing Bz-L-Phe-(^{13}C-Leu) with an equivalent of 1M sodium carbonate followed by lyophilization.

Example 20: Preparation of Bz-Tyr-(^{13}C-Leu)-OMe

[0115] After 1.73 g of 1-^{13}C-L-leucine (Masstrace) was dissolved in hydrogen chloride/methanol and refluxed, the resulting ^{13}C-L-leucine methyl ester was suspended in 100 ml of dichloromethane and 2.00 ml of triethylamine was added dropwise while being ice-

cooled and stirred. Further, 4.05 g of N-benzoyl-L-tyrosine, 4.35 g of HOBt and 100 ml of dichloromethane were added. Then, a solution of 2.73 g of WSC dissolved in 150 ml of dichloromethane was added and stirred for 1 hour while being ice-cooled and then overnight at room temperature. The completion of the reaction was confirmed by silica gel thin layer chromatography using chloroform:methanol (95:5) as a developing solvent. The reaction mixture was concentrated, extracted with ethyl acetate, washed with 1N-HCl, 5% $NaHCO_3$, and water, dried over magnesium sulfate, and concentrated to dryness to yield 5.5 g of N-Bz-Tyr-(^{13}C-Leu)-OMe.

Example 21: Preparation of Bz-Tyr-(^{13}C-Leu) and its sodium salt

[0116] After 5.5 g of N-Bz-Tyr-(13C-Leu)-OMe was dissolved in 150 ml of methanol, 14.6 ml of 1N NaOH was added dropwise while being ice-cooled and stirred followed by heating and stirring at 70°C for 3.5 hours. The completion of the reaction was confirmed by silica gel thin layer chromatography using chloroform:methanol (95:5) as a developing solvent. After the reaction was completed, the reaction mixture was neutralized with 1N-HCl, concentrated and dissolved in 5% $NaHCO_3$. After washing with ethyl acetate, 5% $NaHCO_3$ was acidified with 1N-HCl. The reaction mixture was extracted with ethyl acetate, washed with water, dried over magnesium sulfate, and concentrated to dryness to yield 4.39 g of N-Bz-Tyr-(^{13}C-Leu), which was then recrystallized with ethyl acetate.

[0117] The structure and ^{13}C-labeled position were confirmed by ^{13}C-NMR and mass spectrometry.
^{13}C-NMR (methanol-d4, 300 MHz): 175.9 ppm (^{13}COOH)
Mass spectrometry (m/z): 399 (M^+), 381, 240, 147, 107, 105, 77 LC-MS (m/z): 400 (M^++H), 268, 240

[0118] The sodium salt of N-Bz-Tyr-(^{13}C-Leu) was obtained by neutralizing N-Bz-Tyr-(^{13}C-Leu) with an equivalent of 1M sodium carbonate followed by lyophilization.

Example 22: Preparation of Arg-(^{13}C-Leu)

[0119] One (1) g of 1-^{13}C-L-leucine (Masstrace), 1.7 g of p-toluensulfonic acid monohydrate (TosOH·H_2O) and 3.7 ml of benzyl alcohol (BzlOH) were dissolved in 10 ml of dry benzene and heated and refluxed in an oil bath (110 °C) using a Dean-Stark apparatus equipped with a reflux condenser in an evapolation flask. the reaction was carried out for 5 hours while separating water produced as the reaction proceeded. After the reaction was over, 15 ml of ether and 15 ml of petroleum ether were added to crystallize the reactant and this was recrystallized with ethanol-ether to yield ^{13}C-Leu-OBzl.

[0120] N α-Carbobenzoxy Ng-tosyl arginine (Z-Arg (Tos)) and an equimolar amount of ^{13}C-Leu-OBzl were dissolved in dry tetrahydrofuran (THF) and an equimolar amount of HOBt, two molar amounts of dimethylaminopyridine and 1.5 molar amounts of WSC were added to react for 3 hours. After the reaction was over and the solvent was distilled out under reduced pressure, the material was dissolved in chloroform and the chloroform layer was washed sequentially with 10% citric acid, water, 4% $NaHCO_3$ and water. The chloroform layer was dried over Na_2SO_4 and then the solvent was distilled out. The residue was recrystallized from ethanol-ether to yield Z-Arg(Tos)-^{13}C-Leu-OBzl. Then, 1 g of Z-Arg (Tos)-^{13}C-Leu-OBzl was dissolved in 3.6 ml of thioanisole, 1.5 ml of trifluoroacetic acid (TFA) and 0.6 ml of trifluoromethyl- sulfonic acid (TFMSA) and reacted at room temperature for 2 hours. After distilling out the solvent, the residue was dissolved in water and treated with an anionic exchange resin (AG-X8, acetic acid type). The resulting solution was concentrated and purified in a LH20 column (2.5 cm x 60 cm) equilibrated with methanol:water (1:1) to yield 200 mg of Arg-(^{13}C-Leu).

[0121] The structure and ^{13}C-labeled position were confirmed by ^{1}H-NMR and ^{13}C-NMR.
^{1}H-NMR (DMSO-d6, 400 MHz):

1.073-1.015 ppm 6H: CH- (C$\underline{H}_3$)$_2$ Leu
1.868-1.690 ppm 7H: C$\underline{H}$- (CH_3)$_2$ Leu, CH-C$\underline{H}_2$-CH Leu CH-C$\underline{H}_2$-C$\underline{H}_2$-CH_2 Arg
3.26 ppm 2H: C$\underline{H}_2$-NH-C=NH_2 Arg
3.477 ppm: H_2O
3.649 ppm ^{1}H: NH-C$\underline{H}$-COOH Leu
4.2 ppm ^{1}H: NH_2-C$\underline{H}$-CONH Arg
7.3-6.8 ppm: guanidine group Arg

^{13}C-NMR (DMSO-d6, 400 MHz):

173.3 ppm: NH-CH-(^{13}C-$\underline{C}$OOH) Leu

Example 23: Preparation of Phe-(^{13}C-Leu)

[0122] After 9.96 g of 1-^{13}C-L-leucine (Masstrace) was dissolved in 75 ml of 1N NaOH, a solution of di-t-butyl dicarbonate (Boc_2O) (18.0 g) in acetone (50 ml) was added. Then, 5.21 ml of triethylamine was added dropwise thereto and stirred at room temperature. After one hour, a solution of Boc_2O (9.8 g) in acetone (40 ml) was added and stirred overnight at room temperature. After acetone was distilled out under reduced pressure, 500 ml of ethyl acetate was added, precipitated with 6N HCl, washed with water and dried over anhydrous sodium sulfate. After the desicating agent was filtered out, 10 ml of cyclohexylamine (CHA) was added. After washing with 1N HCl, Boc-(^{13}C-Leu) was extracted with saturated sodium hydrogencarbonate solution and the aqueous layer was acidified with 6N HCl, extracted with ethyl acetate, washed with water and dried over anhydrous sodium sulfate. After concentration under reduced pressure, the residual oily product was dissolved in hexane and 1.2 ml of water was added to crystallize to yield Boc-(^{13}C-Leu)-OH·H_2O. Ten (10) g of Boc-(^{13}C-Leu)-OH·H_2O was dissolved in ethanol (50

ml)-water (20 ml) and 6.52 g of cesium carbonate dissolved in 20 ml of distilled water was added. After concentration under reduced pressure, ethanol and toluene were added and the remaining water was azeotropically removed out to yield a gel product, which was suspended in 300 ml of dimethylformamide (DMF). To the suspension, 5.2 ml of benzyl bromide was dropwise added at room temperature under stirring. After stirring at room temperature for 45 minutes, the solvent was distilled out under reduced pressure. Ethyl acetate was added and washed with water. After drying over anhydrous sodium sulfate, ethyl acetate was distilled out under reduced pressure to yield an oily product. To the product, 59 ml of TFA was added and stirred at room temperature for 40 minutes. After adding 8.37 g of p-toluenesulfonic acid monohydrate, TFA was distilled out under reduced pressure. Diisopropyl ether was added to the residue to crystallize to yield TosOH·H-(^{13}C-Leu)-OBzl. After dissolving 7.89 g of TosOH·H-(^{13}C-Leu)-OBzl, 5.57 g of t-Boc-L-phenylalanine (Boc-Phe) and 2.97 g of HOBt in 80 ml of DMF, 4.03 ml of water soluble carbodiimide (WSCD: 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide) was added dropwise while being ice-cooled and stirred at room temperature for 3 hours. Ethyl acetate and ice water were added and separated into two layers. The organic layer was washed with saturated sodium hydrogen-carbonate, water, 0.1N HCl and water and dried over anhydrous sodium sulfate. After distilling out ethyl acetate under reduced pressure, hexane was added to crystallize to yield Boc-Phe-(^{13}C-Leu)-OBzl. The Boc-Phe-(^{13}C-Leu)-OBzl (5.2 g) was dissolved in 10 ml of acetic acid and 500 mg of 5% palladium carbon (Pd-C) was added. While stirring at room temperature, hydrogen gas was blown thereinto for 1 hour. After the catalyst was filtered out, the mother liquid was concentrated under reduced pressure to about 10 ml. While being ice-cooled, 7.23 ml of 4.6N HCl/dioxane was added and stirred for 10 minutes followed by further stirring at room temperature for 1 hour. After concentration under reduced pressure, ether was added to solidify. The resulting solid was filtered out, washed with ether and dissolved in 100 ml of distilled water. The insolubles were filtered off with a membrane filter and lyophilized. This product was purified in RP-HPLC (YMC-ODS (10 μm), 30 mm x 250 mm, 20 ml/min, aq. CH_3CN (0.05% HCl)) (5%-5%-20%, 0-15-75 minutes) and the main fraction was lyophilized to yield 2.84 g of Phe-(^{13}C-Leu).

[0123] The structure and ^{13}C-labeled position were confirmed by ^{13}C-NMR, mass spectrometry and amino acid analysis.

^{13}C-NMR (DMSO-d6, 270 MHz): 174.3 ppm (^{13}COOH)
MALD-MS (m/z): 280.20 (M^{+}+H)
Amino acid analysis: Phenylalanine 1.00; Leucine 1.01 (hydrolytic conditions: 6N HCl, 110°C, 22 hours)

Example 24: Preparation of Bz-Ala-Ala-Ala-Ala-Gly-Phe-(^{13}C-Leu) and its sodium salt

[0124] To 33.8 g of N-t-Boc-phenacyl-L-alanine (Boc-Ala-OPac), 9.6 ml of 4.6N HCl/dioxane solution was added and stirred at room temperature for 40 minutes. Diethyl ether was added to solidify and the solid was filtered out and dried under reduced pressure. This material was suspended in 100 ml of dichloromethane and 18.9 g of N-t-Boc-L-alanine (Boc-Ala) was added and cooled to 0°C. After adding 19.2 ml of WSCD, the mixture was stirred at room temperature for 1.5 hours. After concentration under reduced pressure, the residue was dissolved in ethyl acetate/water and the organic layer was washed with water, 1N HCl and water and dried over anhydrous sodium sulfate. Ethyl acetate was distilled out under reduced pressure and diisopropyl ether was added to solidify. The resulting solid was filtered out and recrystallized with acetone-ether-diisopropyl ether to yield Boc-Ala-Ala-OPac.

[0125] To 15.1 g of Boc-Ala-Ala-OPac, 88.8 ml of TFA was added and stirred at room temperature for 50 minutes. After concentration under reduced pressure, 11.3 ml of 4.6N HCl/dioxane solution was added. Diethyl ether/diisopropyl ether was added to solidify and the resulting solid was filtered out and dried under reduced pressure. This material was dissolved in 100 ml of DMF and 7.95 g of Boc-Ala and 5.95 g of HOBt were added. After cooling to 0°C and adding 8.1 ml of WSCD, the mixture was stirred at room temperature for 4 hours. The reaction mixture was cooled and water was added to precipitate. The precipitated solid was filtered out and dissolved in chloroform/methanol (3:1). The organic layer was washed with water and directly concentrated under reduced pressure. Ether was added to crystallize to yield Boc-Ala-Ala-Ala-OPac.

[0126] Boc-Ala-Ala-Ala-OPac (8.99 g) was dissolved in 50 ml of dichloromethane/trifluoroethanol (TFE) (3:1) and to this 100 ml of acetic acid was added. Then, 26.2 g of zinc powder was added and vigorously stirred at 35°C for 1 hour. The insolubles were filtered out and the remaining was concentrated under reduced pressure. Ethyl acetate was added, washed with water and dried over anhydrous sodium sulfate. Ethyl acetate was distilled out under reduced pressure and ether was added to the residue to crystallize to yield Boc-Ala-Ala-Ala-OH.

[0127] After 9.96 g of 1-^{13}C-L-leucine (Masstrace) was dissolved in 75 ml of 1N NaOH, a solution of Boc_2O (18 g) in acetone (50 ml) was added. Then, 5.21 ml of triethylamine was added dropwise thereto and stirred at room temperature. After one hour, a solution of Boc_2O (9.8 g) in acetone (40 ml) was added and stirred overnight at room temperature. After acetone was distilled out under reduced pressure, 500 ml of ethyl acetate was added, precipitated with 6N HCl, washed with water and dried over anhydrous sodium sulfate. After the desiccating agent was filtered out, 10 ml of CHA was added. After washing with 1N HCl, Boc-(^{13}C-Leu) was extracted

with saturated sodium hydrogencarbonate solution and the aqueous layer was acidified with 6N HCl, extracted with ethyl acetate, washed with water and dried over anhydrous sodium sulfate. After concentration under reduced pressure, the residual oily product was dissolved in hexane and 1.2 ml of water was added to crystallize to yield Boc- (^{13}C-Leu)-OH·H_2O.

[0128] Ten. (10) g of Boc-(^{13}C-Leu)-OH·H_2O was dissolved in ethanol (50 ml)-water (20 ml) and 6.52 g of cesium carbonate dissolved in 20 ml of distilled water was added. After concentration under reduced pressure, ethanol and toluene were added and the remaining water was azeotropically removed out to yield a gel product, which was suspended in 300 ml of DMF. To the suspension, 5.2 ml of benzyl bromide was added dropwise at room temperature while being stirred. After stirring at room temperature for 45 minutes, the solvent was distilled out under reduced pressure. Ethyl acetate was added and washed with water. After drying over anhydrous sodium sulfate, ethyl acetate was distilled out under reduced pressure to yield an oily product. To the product, 59 ml of TFA was added and stirred at room temperature for 40 minutes. After adding 8.37 g of p-toluenesulfonic acid monohydrate, TFA was distilled out under reduced pressure. Diisopropyl ether was added to the residue to crystallize to yield TosOH-H-(^{13}C-Leu) -OBzl.

[0129] After dissolving 7.89 g of TosOH·H- (^{13}C-Leu) -OBzl, 5.57 g of Boc-Phe and 2.97 g of HOBt in 80 ml of DMF, 4.03 ml of WSCD was added dropwise while being ice-cooled and the mixture was stirred at room temperature for 3 hours. Ethyl acetate and ice water were added which resulted in two separate layers. The organic layer was washed with saturated sodium hydrogencarbonate, water, 0.1N HCl and water and dried over anhydrous sodium sulfate. After distilling out ethyl acetate under reduced pressure, hexane was added to crystallize to yield Boc-Phe-(^{13}C-Leu)-OBzl.

[0130] To 3.71 g of Boc-Phe-(^{13}C-Leu)-OBzl, 17.5 ml of TFA was added, stirred at room temperature for 40 minutes and concentrated under reduced pressure. After adding 2.2 ml of 4.6N HCl/dioxane, diisopropyl ether was added to crystallize. The crystal was filtered out, dried under reduced pressure and dissolved in 50 ml of DMF and 2.13 g of Boc-Ala-Gly-OH·H_2O and 1.15 g of HOBt were added. While being ice-cooled, 1.56 ml of WSCD was added dropwise, and the mixture was stirred at room temperature for 3 hours. After cooling the reaction mixture, the precipitated solid was filtered out and washed with water. This material was dissolved in ethyl acetate and dried over anhydrous sodium sulfate. After concentration under reduced pressure, the material was crystallized with diisopropyl ether to yield Boc-Ala-Gly-Phe-(^{13}C-Leu)-OBzl.

[0131] To 3.59 g of Boc-Ala-Gly-Phe-(^{13}C-Leu)-OBzl, 22.2 ml of TFA was added and stirred at room temperature for 45 minutes. After concentration under reduced pressure, 1.7 ml of 4.6N HCl/dioxane was added and solidified with diisopropyl ether. The solid was filtered out and dissolved in 50 ml of DMF and 2.03 g of Boc-Ala-Ala-Ala-OH and 1.06 g of 3-hydroxy-4-oxo-3,4-dihydro-1,2,3-benzotriazine (HOObt) were added. While being ice-cooled 1.19 ml of WSCD was added dropwise and the mixture was stirred at room temperature for 4 hours. After cooling the reaction mixture, water was added and the precipitated solid was filtered out and washed with water. The solid was suspended in methanol, filtered out and dried under reduced pressure. The material was dissolved in 100 ml of chloroform/trifluoroethanol and impurities were filtered out. The solvent was removed out under reduced pressure and ether was added to the residue followed by filtering to yield Boc-Ala-Ala-Ala-Ala-Gly-Phe-(^{13}C-Leu)-OBzl.

[0132] To 2.68 g of Boc-Ala-Ala-Ala-Ala-Gly-Phe-(^{13}C-Leu)-OBzl, 19 ml of TFA was added and stirred at room temperature for 45 minutes. After concentration under reduced pressure, ether was added to solidify. The solid was filtered out and dissolved in 20 ml of DMF and 0.80 g of benzoyl 1-hydroxysuccinimmide (Bz-ONSu) was added. After adding dropwise 0.5 ml of triethylamine, the mixture was stirred at room temperature for 1 hour. Then, 20 ml of DMF was further added and triethylamine was added to adjust the pH to 6. The mixture was stirred at room temperature for additional 3 hours. After cooling the reaction mixture, water was added and the precipitated solid was filtered out and washed with water. The solid was suspended in methanol and filtered out to yield Bz-Ala-Ala-Ala-Ala-Gly-Phe-(^{13}C-Leu)-OBzl.

[0133] After dissolving 1.63 g of Bz-Ala-Ala-Ala-Ala-Gly-Phe- (^{13}C-Leu)-OBzl in 80 ml of dichloromethane/hexafluoroisopropyl alcohol (3:1), 0.32 g of 5% palladium-carbon suspended in water/methanol was added. The reaction mixture was stirred at 27°C for 4 hours while introducing thereinto hydrogen gas. A powdery filter paper was used to filter out the catalyst and the filtrate was concentrated under reduced pressure. Water was added to solidify and the solid was filtered out and washed with water. The solid was suspended in methanol, filtered out and dried under reduced pressure. This material was dissolved in 50 ml of hexafluoroisopropyl alcohol and concentrated under reduced pressure and ethyl acetate was added to the residue to crystallize to yield 1.19 g of Bz-Ala-Ala-Ala-Ala-Gly-Phe-(^{13}C-Leu).

[0134] The structure and ^{13}C-labeled position were confirmed by ^{13}C-NMR, mass spectrometry and amino acid analysis.

^{13}C-NMR (DMSO-d6, 270 MHz): 173.8 ppm (^{13}COOH)

PD-MS (m/z): 725.3 (M^++H), 748.2 (M^++Na), 764.4 (M^++K)

Amino acid analysis: Phenylalanine 1.00; Glycine 1.08; Alanine 4.09; Leucine 1.04 (hydrolytic conditions: 6N HCl, 110°C, 22 hours)

[0135] The sodium salt of Bz-Ala-Ala-Ala-Ala-Gly-Phe-(^{13}C-Leu) was obtained by neutralizing Bz-Ala-Ala-Ala-Ala-Gly-Phe-(^{13}C-Leu) with an equivalent of 1M so-

dium carbonate followed by lyophilization.

Example 25: Preparation of Boc-Ala-Ala-Ala-Ala-Gly-Phe-(^{13}C-Leu) and its sodium salt

[0136] As in Example 24, Boc-Ala-Ala-Ala-Ala-Gly-Phe-(^{13}C-Leu)-OBzl was obtained. After dissolving 1.62 g of Boc-Ala-Ala-Ala-Ala- Gly-Phe-(^{13}C-Leu)-OBzl in 40 ml of dichloromethane/hexafluoroisopropyl alcohol (3: 1), 0.16 g of 5% palladium-carbon suspended in water/ methanol was added. Hydrogen gas was introduced into the reaction mixture for 1.5 hours while being stirred at 27°C. A powdery filter paper was used to filter out the catalyst and the filtrate was concentrated under reduced pressure. Acetonitrile was added to solidify and the solid was filtered out. The solid was dried under reduced pressure and dissolved in 30 ml of hexafluoroisopropyl alcohol. The insolubles were filtered out and the remainder was concentrated under reduced pressure. Ethyl acetate was added to the residue and the precipitated gel material was filtered to yield 1.56 g of Boc-Ala-Ala-Ala-Ala-Gly-Phe-(^{13}C-Leu).

[0137] The structure and ^{13}C-labeled position were confirmed by ^{13}C-NMR, mass spectrometry and amino acid analysis.

^{13}C-NMR (DMSO-d6, 270 MHz): 173.7 ppm (^{13}COOH)

PD-MS (m/z): 721.7 (M^{+}+H), 743.9 (M^{+}+Na), 759.9 (M^{+}+K)

Amino acid analysis: Phenylalanine 1.00; Glycine 1.09; Alanine 4.07; Leucine 1.04 (hydrolytic conditions: 6N HCl, 110°C, 22 hours)

[0138] The sodium salt of Boc-Ala-Ala-Ala-Ala-Gly-Phe-(^{13}C-Leu) was obtained by neutralizing Boc-Ala-Ala-Ala-Ala-Gly-Phe-(^{13}C-Leu) with an equivalent of 1M sodium carbonate followed by lyophilization.

Example 26: Preparation of Bz-Ala-Ala-Ala-Ala-(^{13}C-Gly)-Phe-Leu and its sodium salt

[0139] After dissolving 5 g of 1-^{13}C-glycine (Masstrace) in aqueous solution (17.5 ml) of sodium hydroxide (2.56 g) and adding 3.65 ml of triethylamine, a solution of Boc_2O (15.1 ml) in acetone (8 ml) was added and stirred at room temperature for 17 hours. After concentration under reduced pressure, citric acid was added to the aqueous layer to adjust the pH to 4 and sodium chloride was added to salt out followed by extraction with ethyl acetate. The extract was directly dried over magnesium sulfate and the desicating agent was filtered out. After adding 6.76 ml of CHA, the filtrate was allowed to stand overnight in a refrigerator. The precipitated crystal was filtered out to yield Boc- (^{13}C-Gly)-OH·CHA.

[0140] Eight (8) g of Boc-(^{13}C-Gly)-OH·CHA was suspended in 50 ml of THF and 6.32 ml of 4.6N HCl/dioxane was added. The mixture was fully disrupted in a ultrasonic washer and ether was added. The precipitated solid was filtered out and the filtrate was concentrated under reduced pressure. Ether was added to the residue and the insolubles were filtered and concentrated under reduced pressure. The residue was washed with hexane and filtered out to yield Boc-(^{13}C-Gly)-OH.

[0141] After dissolving 5.31 g of Boc-Phe, 7.87 g of TosOH·H-Leu-OBzl and 2.84 g of HOBt in 40 ml of DMF, 3.72 ml of WSCD was added under ice-cooling and stirred for 30 minutes and at room temperature for further 2.5 hours. After concentration under reduced pressure, ethyl acetate was added, washed sequentially with saturated aqueous sodium hydrogencarbonate solution, saturated aqueous sodium chloride solution, 10% aqueous citric acid solution and saturated aqueous sodium chloride solution, and dried over magnesium sulfate. After concentration under reduced pressure, 43 ml of 4.6N HCl/dioxane was added and allowed to stand at room temperature for 30 minutes. After concentration under reduced pressure, ether was added and the precipitated solid was filtered out to yield HCl·H-Phe-Leu-OBzl.

[0142] After dissolving 3.52 g of Boc-(^{13}C-Gly)-OH, 8.88 g of HCl·H-Phe-Leu-OBzl and 2.70 g of HOBt in 40 ml of DMF, 3.54 ml of WSCD was added under ice-cooling and stirred for 30 minutes and at room temperature for further 14 hours. After concentration under reduced pressure, the material was dissolved in ethyl acetate, washed sequentially with saturated aqueous sodium hydrogencarbonate solution, saturated aqueous sodium chloride solution, 10% aqueous citric acid solution and saturated aqueous sodium chloride solution, and dried over magnesium sulfate. After concentration under reduced pressure, Boc- (^{13}C-Gly)-Phe-Leu-OBzl was obtained.

[0143] To 10.4 g of Boc-(^{13}C-Gly)-Phe-Leu-OBzl, 42.8 ml of 4.6N HCl/dioxane was added and allowed to stand at room temperature for 45 minutes. After concentration under reduced pressure, an oily product was obtained. The oily product was dissolved in 40 ml of DMF and 3.7 g of Boc-Ala and 2.66 g of HOBt were added followed by adding 3.49 ml of WSCD while being ice-cooled and this was stirred for 30 minutes and for 16 hours further at room temperature. After concentration under reduced pressure, the material was dissolved in ethyl acetate and washed with saturated aqueous sodium hydrogencarbonate solution, saturated aqueous sodium chloride solution, 10% aqueous citric acid solution and saturated aqueous sodium chloride solution. The organic layer was concentrated under reduced pressure and the residue was washed with diisopropyl ether and filtered out to yield Boc-Ala-(^{13}C-Gly)-Phe-Leu-OBzl.

[0144] As in Example 24, Boc-Ala-Ala-Ala-OH was obtained.

[0145] To 3.59 g of Boc-Ala-(^{13}C-Gly)-Phe-Leu-OBzl, 22.2 ml of TFA was added and allowed to stand at room temperature for 45 minutes. After concentration under reduced pressure, 1.7 ml of 4.6N HCl/dioxane was added and diisopropyl ether was added to solidify. The solid was filtered out and dried. This material was dissolved in 50 ml of DMF and 2.03 g of Boc-Ala-Ala-Ala-OH and

1.06 g of HOObt were added. While being ice-cooled 1.19 ml of WSCD was added and this was stirred for 30 minutes and for 14 hours further at room temperature. After cooling the reaction mixture, water was added and the precipitated gel was filtered out to yield Boc-Ala-Ala-Ala-Ala- (^{13}C-Gly)-Phe-Leu-OBzl.

[0146] To a suspension of 2.00 g of Boc-Ala-Ala-Ala-Ala-(^{13}C-Gly)-Phe-Leu-OBzl and 1.34 ml of anisole, 12 ml of anhydrous hydrogen fluoride was introduced while being stirred and cooled in a dry ice-methanol bath. The material was stirred for 1 hour under cooling at -5°C and hydrogen fluoride was distilled out at this temperature. Ether was added to the residue and the precipitated solid was filtered out. The solid was suspended into 40 ml of DMF-water (9:1) and 704 mg of Bz-ONSu and 1.03 ml of triethylamine were added and stirred at room temperature for 6 hours. Then, 40 ml of DMF-water (9:1) was added and stirred overnight at room temperature. Further, 541 mg of Bz-ONSu was added and stirred overnight at room temperature. After concentration under reduced pressure, ethyl acetate was added to the residue, fully disrupted in a ultrasonic washer and filtered out to yield 1.53 g of Bz-Ala-Ala-Ala-Ala-(^{13}C-Gly) -Phe-Leu.

[0147] The structure and ^{13}C-labeled position were confirmed by ^{13}C-NMR, mass spectrometry and amino acid analysis.

^{13}C-NMR (DMSO-d6, 270 MHz) : 168.2 ppm (^{13}COOH)

PD-MS (m/z): 725.5 (M^{+}+H), 747.8 (M^{+}+Na), 764.3 (M^{+}+K)

Amino acid analysis: Phenylalanine 1.00; Glycine 1.14; Alanine 4.25; Leucine 1.07 (hydrolytic conditions: 6N HCl, 110°C, 22 hours)

[0148] The sodium salt of Bz-Ala-Ala-Ala-Ala-(^{13}C-Gly)-Phe-Leu was obtained by neutralizing Bz-Ala-Ala-Ala-Ala-(^{13}C-Gly)-Phe-Leu with an equivalent of 1M sodium carbonate followed by lyophilization.

Example 27: Preparation of Bz-Tyr-O-(^{13}C-Et)

[0149] After 5.31 g of N-t-Boc-O-benzyl-L-tyrosine and 3.5 g of 1,2-^{13}C-ethanol (Masstrace) were dissolved in 30 ml of DMF, 2.32 g of HOBt was added and 3.14 ml of WSCD was dropwise added under ice-cooling followed by stirring at room temperature for 4 hours. Ethyl acetate was added and the material was washed sequentially with water, saturated aqueous sodium hydrogencarbonate solution, saturated aqueous sodium chloride solution, 1N HCl and saturated aqueous sodium chloride solution, and dried over anhydrous sodium sulfate. The solvent was distilled out under reduced pressure. While cooled at -10°C, 20 ml of TFA was added to the resulting colorless oily product followed by stirring for 10 minutes and for 50 minutes further at room temperature. After concentration under reduced pressure, 3.73 ml of 4.6N HCl/dioxane was added and diisopropyl ether was added to solidify. The residue was filtered out and washed further with diisopropyl ether. This residue was dissolved in 50 ml of DMF and 1.99 ml of benzoyl chloride and 4.00 ml of triethylamine were added while cooled at -10°C. After stirring for 30 minutes and further at room temperature for 3 hours, ethyl acetate was added and washed sequentially with saturated aqueous sodium chloride solution, saturated aqueous sodium hydrogencarbonate solution, saturated aqueous sodium chloride solution, 1N HCl and saturated aqueous sodium chloride solution, and dried over anhydrous sodium sulfate. The solvent was distilled out under reduced pressure and the residue was washed with ether and dried under reduced pressure. This material was recrystallized from methanol to yield Bz-Tyr(Bzl) -O-(^{13}C-Et). Then, 800 mg of Bz-Tyr(Bzl)-O-(^{13}C-Et) was dissolved in 30 ml of acetic acid and 1600 mg of palladium black was added. Hydrogen gas was introduced for 6 hours while vigorously stirred at room temperature. Hydrogen gas was stopped and the reaction mixture was stirred overnight at room temperature. A powdery filter paper was used to filter out the catalyst and then acetic acid was distilled out under reduced pressure. Hexane was added and concentration under reduced pressure gave a colorless solid. Water was added and the supernatant was repeatedly removed out by decantation. The material was dissolved in 20% aqueous acetonitrile solution followed by lyophilization. This material was subjected to RP-HPLC (YMC A-323 ODS, 30 x 250 mm, 20-50% aq. CH_3CN containing 0.1% TFA (60 minutes), flow rate 20 ml/min) to take a main fraction which was then lyophilized to yield 350 mg of Bz-Tyr-O-(^{13}C-Et).

[0150] The structure and ^{13}C-labeled position were confirmed by ^{13}C-NMR and mass spectrometry.

^{13}C-NMR ($CDCl_3$, 270 MHz): 14.2 ppm ($^{13}CH_3$), 61.6 ppm (^{13}CH2)

PD-MS (m/z) : 316.2 (M^{+}+H)

Example 28: Preparation of Bz-Arg-(^{13}C-Leu)·HCl

[0151] As in Example 24, TosOH-H-(^{13}C-Leu)-OBzl was obtained. After 18.1 g of TosOH-H-(^{13}C-Leu)-OBzl, 14.5 g of N-t-Boc-arginine hydrochloride monohydrate (Boc-Arg·HCl·H_2O) and 5.95 g of HOBt were dissolved in 130 ml of DMF, 8.4 ml of WSCD was added while being cooled at -20 °C and the mixture was stirred at room temperature for 3.5 hours. After DMF was distilled off, the material was dissolved in ethyl acetate and washed with saturated aqueous sodium hydrogencarbonate solution, saturated aqueous sodium chloride solution, 0.1 N HCl and saturated aqueous sodium chloride solution. The material was dried over anhydrous sodium sulfate. After ethyl acetate was distilled off under reduced pressure, hexane was added to yield a crystal. The crystal was filtered out, washed with hexane and dried under reduced pressure. The resultant crude crystal was dissolved in 50 ml of acetonitrile. To this solution, diisopropyl ether was added to yield a crystal of Boc-Arg-(^{13}C-Leu)-OBzl·TosOH.

[0152] To 24.8 g of Boc-Arg-(^{13}C-Leu)-OBzl·TosOH, 50 ml of TFA was added and the mixture was stirred at room temperature for 30 minutes and concentrated under reduced pressure. After 20 ml of 4.6N HCl/dioxane was added, ether was added to yield a crystal of a hydrochloride. The hydrochloride was filtered out and dried under reduced pressure. To the resultant hydrochloride, 5.12 g of benzoic acid and 5.67 g of HOBt were added and the mixture was dissolved in 110 ml of DMF. To this solution, 7.7 ml of WSCD was added while being cooled at -20 °C and the mixture was stirred at room temperature for 15 hours. The reaction solution was concentrated, dissolved in ethyl acetate and washed with 0.1 N HCl, saturated aqueous sodium chloride solution and saturated aqueous sodium hydrogencarbonate solution to yield a crystal. The crystal was filtered out, washed with ethyl acetate and water, and dissolved in acetic acid. To this solution, ether was added to yield a colorless oily product. The product was dissolved in 250 ml of acetic acid. To this solution, 3 g of palladium-carbon was added. The mixture was stirred at room temperature while introducing hydrogen gas thereinto for 2 hours. The catalyst was filtered off and acetic acid was distilled off. The residue was dissolved in 9 ml of 4.6 N HCl/dioxane. Diisopropyl ether was added to yield a crystal of 16.4 g of Bz-Arg-(^{13}C-Leu)·HCl.

[0153] The structure and ^{13}C-labeled position were confirmed by ^{13}C-NMR,
mass spectrometry and amino acid analysis.
^{13}C-NMR (D_2O, 270 MHz): 177.5 ppm (^{13}COOH)
ESI-MS (m/z): 393.2 (M^++H)
Amino acid analysis: Arginine: 1.00; Leucine 1.02 (hydrolytic conditions: 6N HCl, 110°C, 22 hours)

Example 29: Bz-L-Phe-(^{13}C-Leu) breath test

[0154] As in Example 15-1, Bz-L-Phe-(^{13}C-Leu) breath test was carried out wherein 250 mg/kg of Bz-L-Phe-(^{13}C-Leu)·Na was orally administered to the chronic pancreatitis rats (n=4) and the normal rats (n=4) and the degree of increase (Δ^{13}C(‰)) of the $^{13}CO_2$ concentration in the CO_2 in the breath after the administration was measured. The Δ^{13}C (‰) value at 10 minutes after the administration was 6.97 ± 6.09 ‰ in the chronic pancreatitis rats and 115.02 ± 71.26 ‰ in the normal rats; thus, the value of the chronic pancreatitis rats was significantly smaller than the normal rats ($p<0.05$ (ANOVA with Fischer LSD)) (Table 1).

Table 1

Bz-L-Phe-(^{13}C-Leu) breath test	
	Δ^{13}C (‰) at 10 min
Chronic pancreatitis #1	1.61
Chronic pancreatitis #2	3.28
Chronic pancreatitis #3	17.21
Chronic pancreatitis #4	5.77
Normal #1	31.29
Normal #2	60.25
Normal #3	165.37
Normal #4	203.16

[0155] Bz-L-Phe-(^{13}C-Leu)-Na was orally administered in an amount of 250 mg/kg to the chronic pancreatitis rats (n=4) and the normal rats (n=4).

Example 30: Bz-Tyr-(^{13}C-Leu) breath test

[0156] As in Example 15-1, Bz-Tyr-(^{13}C-Leu) breath test was carried out wherein 250 mg/kg of Bz-Tyr-(^{13}C-Leu)·Na was orally administered to the chronic pancreatitis rats (n=4) and the normal rats (n=4) and the degree of increase (Δ^{13}C (‰)) of the $^{13}CO_2$ concentration in the exhaled CO_2 after the administration was measured. The Δ^{13}C (‰) value at 20 minutes after the administration was 3.66 ± 3.24 ‰ in the chronic pancreatitis rats and 69.53 ± 32.50 ‰ in the normal rats; thus, the value of the chronic pancreatitis rats was significantly smaller than the normal rats ($p<0.05$ (ANOVA with Fischer LSD)) (Table 2).

Table 2

Bz-Tyr-(^{13}C-Leu) breath test	
	Δ^{13}C (‰) at 20 min
Chronic pancreatitis #1	8.76
Chronic pancreatitis #2	3.58
Chronic pancreatitis #3	2.44
Chronic pancreatitis #4	-0.15
Normal #1	111.32
Normal #2	65.76
Normal #3	21.02
Normal #4	80.02

[0157] Bz-Tyr-(^{13}C-Leu)·Na was orally administered in an amount of 250 mg/kg to the chronic pancreatitis rats (n=4) and the normal rats (n=4).

Example 31: Arg-(^{13}C-Leu) breath test

[0158] As in Example 15-1, Arg-(^{13}C-Leu) breath test was carried out wherein 30 mg/kg of Arg-(^{13}C-Leu) was orally administered to the chronic pancreatitis rats (n=4) and the normal rats (n=4) and the degree of increase

($\Delta^{13}C$ (‰)) of the $^{13}CO_2$ concentration in the exhaled CO_2 after the administration was measured. The $\Delta^{13}C$ (‰) value at 30 minutes after the administration was 4.37 ± 1.83 ‰ in the chronic pancreatitis rats and 12.37 ± 2.26 ‰ in the normal rats; thus, the value of the chronic pancreatitis rats was significantly smaller than the normal rats ($p<0.01$ (ANOVA with Fischer LSD)) (Table 3).

Table 3

Arg-(^{13}C-Leu) breath test	
	$\Delta^{13}C$ (‰) at 30 min
Chronic pancreatitis #1	3.74
Chronic pancreatitis #2	6.97
Chronic pancreatitis #3	4.87
Chronic pancreatitis #4	1.92
Normal #1	10.30
Normal #2	13.74
Normal #3	15.37
Normal #4	10.08

[0159] Arg-(^{13}C-Leu) was orally administered in an amount of 30 mg/kg to the chronic pancreatitis rats (n=4) and the normal rats (n=4).

Example 32: Bz-Ala-Ala-Ala-Ala-(^{13}C-Gly)-Phe-Leu breath test

[0160] As in Example 15-1, Bz-Ala-Ala-Ala-Ala-(^{13}C-Gly)-Phe-Leu breath test was carried out wherein 420 mg/kg of Bz-Ala-Ala-Ala-Ala-(^{13}C-Gly)-Phe-Leu·Na was orally administered to the chronic pancreatitis rats (n=2) and the normal rats (n=2) and the degree of increase ($\Delta^{13}C$ (‰)) of the $^{13}CO_2$ concentration in the exhaled CO_2 after the administration was measured. The $\Delta^{13}C$ (‰) (mean) value at 15 minutes after the administration was 0.61 ‰ in the chronic pancreatitis rats and 33.32 %o in the normal rats; thus, the value of the chronic pancreatitis rats was smaller than the normal rats (Table 4).

Table 4

Bz-Ala-Ala-Ala-Ala-(^{13}C-Gly)-Phe-Leu breath test	
	$\Delta^{13}C$ (‰) at 15 min
Chronic pancreatitis #1	-1.90
Chronic pancreatitis #2	3.12
Normal #1	23.47
Normal #2	43.16

[0161] Bz-Ala-Ala-Ala-Ala-(^{13}C-Gly)-Phe-Leu-Na was orally administered in an amount of 420 mg/kg to the chronic pancreatitis rats (n=2) and the normal rats (n=2).

Example 33: Bz-Arg-(^{13}C-Leu) breath test

[0162] As in Example 15-1, Bz-Arg-(^{13}C-Leu) breath test was carried out wherein 100 mg/kg of Bz-Arg-(^{13}C-Leu) · HCl was orally administered to the chronic pancreatitis rats (n=3) and the normal rats (n=3) and the degree of increase ($\Delta^{13}C$ (‰)) of the $^{13}CO_2$ concentration in the exhaled CO_2 after the administration was measured. The $\Delta^{13}C$ (‰) value at 20 minutes after the administration was 3.05 ± 6.97 ‰ in the chronic pancreatitis rats and 68.77 ± 12.01 ‰ in the normal rats; thus, the value of the chronic pancreatitis rats was significantly smaller than the normal rats ($p<0.01$ (ANOVA with Fischer LSD)) (Table 5).

Table 5

Bz-Arg-(^{13}C-Leu) breath test	
	$\Delta^{13}C$ (‰) at 20 min
Chronic pancreatitis #1	3.69
Chronic pancreatitis #2	11.25
Chronic pancreatitis #3	-5.79
Normal #1	51.94
Normal #2	74.46
Normal #3	79.91

Formulation Example 1: (Liquid for internal use)

[0163] Purified water was added to 5 parts by weight of Bz-DL-Phe-(^{13}C-Leu)·Na to produce a total of 100 parts by weight and this total amount was dissolved and sterilized through a Millipore filter. The filtrate was taken into a vial bottle and sealed to yield a liquid for internal use.

Advantages of the Invention:

[0164] The present invention provides a highly sensitive pancreatic exocrine function test method which imparts low stress on a subject and gives accurate results soon.

[0165] Conventional simple tests for pancreatic exocrine function are less sensitive and therefore have become less used as diagnostic tests for pancreatitis and currently have been utilized generally for follow up of prognosis of pancreatitis which always necessitates repeated tests. However, a highly sensitive simple test for pancreatic exocrine function would be often utilized in diagnosing for pancreatitis in a physical examination. Further, it could be applied to assessing the seriousness of chronic pancreatitis, precognition of onset of serious fulminant pancreatitis with a still high mortality (30%), diagnosis of causes for pancreatitis, and early diagnosis

of pancreatic cancer. It would also be usefull as a diagnostic method for ruling out pancreatitis in medical examination of general outpatients.

Claims

1. Use of an amino acid or a peptide containing at least one ^{13}C or ^{14}C atom, or a pharmaceutically acceptable salt thereof other than Bz-Tyr-^{13}C-PABA in the manufacture of a diagnostic agent for pancreatic exocrine function, wherein the diagnostic agent is used in a breath test.

2. Use according to claim 1 wherein the amino acid molecule contains a ^{13}C or ^{14}C atom.

3. Use according to claim 1 wherein the amino acid or peptide has a modifying or protecting group and the modifying or protecting group contains a ^{13}C or ^{14}C atom.

4. Use according to any one of claims 1 to 3, wherein the amino acid or peptide is represented by the following formula (I):

$$X_1\text{-}R_1\text{-}Y_1 \qquad \text{(I)}$$

wherein X_1 is a hydrogen atom or a protecting group,
R_1 is a peptide of 2 to 50 amino acids, an amino acid or a single bond,
Y_1 is an amino acid optionally having a protecting group.

5. Use according to claim 4, wherein at least one of the amino acids in R_1 and Y_1, or at least an ester group in Y_1 when the amino acid in Y_1 is protected with the ester group, is ^{13}C- or ^{14}C-labelled.

6. Use according to any one of the preceding claims wherein the amino acid or peptide, or pharmaceutically acceptable salt thereof is capable of being a substrate of a protease or proteases such that it is subsequently capable of being decarboxylated to generate $^{13}CO_2$ or $^{14}CO_2$.

7. Use according to claim 6, wherein the protease or proteases are pancreatic exocrine proteases.

8. Use according to claim 7, wherein the pancreatic exocrine protease or proteases are selected from the group consisting of chymotrypsin, trypsin, elastase, and carboxypeptidases.

9. A ^{13}C- or ^{14}C-labelled compound or salt thereof for use in diagnosis
wherein the ^{13}C- or ^{14}C-labelled compound is represented by the following formula (II):

$$X_2\text{-}R_2\text{-}Y_2\text{-}Z_1 \qquad \text{(II)}$$

wherein X_2 is a hydrogen atom or a protecting group,
R_2 is a peptide of 2 to 5 amino acids, an amino acid or a single bond,
Y_2 is an amino acid,
Z_1 is an amino acid optionally having a protecting group, and at least one of the amino acids in R_2,Y_2 and Z_1, or at least one of the protecting groups in X_2 and Z_1 when the protecting groups contain a carbon atom, is ^{13}C or ^{14}C-labelled,
and wherein

1) in the case where X_2 is a protecting group and R_2 is a single bond,

1-i) when Z_1 is D-Ala, Y_2 is an amino acid other than D-Ala,
1-ii) when Z_1 is Ala-OMe, Y_2 is an amino acid other than Leu,
1-iii) when Z_1 is Pro-OMe, Y_2 is an amino acid other than Gly,

2) in the case where X_2 is a protecting group and R_2 is an amino acid,

2-i) when Z_1 is D-Ala, Y_2 is an amino acid other than Val,
2-ii) when Z_1 is Ala, Y_2 is an amino acid other than Leu,
2-iii) when Z_1 is Gly-OEt, Y_2 is an amino acid other than Pro,

3) in the case where X_2 is a protecting group, R_2 is a peptide of 2 amino acids and Z_1 is Gln optionally having SEt, Y_2 is an amino acid other than Ala,
4) in the case where X_2 is a hydrogen atom and R_2 is a single bond,

4-i) when Z_1 is Pro,Y_2 is an amino acid other than Ala,
4-ii) when Z_1 is Leu , Y_2 is an amino acid other than Gly,Val,Leu and Tyr,
4-iii) when Z_1 is Phe, Y_2 is an amino acid other than Gly,
4-iv) when Z_1 is Gly-OEt, Y_2 is an amino acid other than Gly,
4-v) when Z_1 is Ala-OMe, Y_2 is an amino acid other than Leu,

5) in the case where X_2 is a hydrogen atom and R_2 is an amino acid,

5-i) when Z_1 is Leu, Y_2 is an amino acid other than Pro,
5-ii) when Z_1 is Phe, Y_2 is an amino acid other than Pro,
5-iii) when Z_1 is Gly, Y_2 is an amino acid other than Gly,
5-iv) when Z_1 is Pro, Y_2 is an amino acid other than Leu,
5-v) when Z_1 is Met, Y_2 is an amino acid other than Asp,
5-vi) when Z_1 is Asn-Bzl, Y_2 is an amino acid other than Leu,

6) in the case where X_2 is a hydrogen atom and R_2 is a peptide of 2 amino acids,

6-i) when Z_1 is Leu, Y_2 is an amino acid other than Leu,
6-ii) when Z_1 is Ser, Y_2 is an amino acid other than Asp, and

7) in the case where X_2 is a hydrogen atom and R_2 is a peptide of 3 amino acids,

7-i) when Z_1 is Leu, Y_2 is an amino acid other than Phe,
7-ii) when Z_1 is Met, Y_2 is an amino acid other than Phe;

provided that the following compounds are excluded
Ala-Pro, Gly-Leu, Gly-Phe, Val-Leu, Leu-Leu, Tyr-Leu, Ac-D-Ala-D-Ala, Gly-Gly-OEt, Leu-Ala-OMe, Ac-Gly-Pro-OMe, Boc-Leu-Ala-OMe, Gly-Pro-Leu, Gly-Pro-Phe, Ala-Gly-Gly, Gly-Leu-Pro, Phe-Asp-Met, Gly-Leu-Pro, Dansyl-Tyr-Val-D-Ala, Cbz-Gly-Leu-Ala, Thr-Leu-Asn-Bzl, Z-Pro-Pro-Gly-OEt, Leu-Leu-Leu-Leu, Lys-Arg-Asp-Ser, Ac-Leu-Ala-Ala-Gln (NMe_2), Ac-Leu-Ala-Ala-Gln (NMe_2)-SEt, Tyr-Gly-Gly-Phe-Leu and Tyr-Gly-Gly-Phe-Met, wherein Ac is acetyl, Et is ethyl, Me is methyl, Boc is t-butyloxycarbonyl, Dansyl is dansyl, Cbz is carbobenzyloxy, Bzl is benzoyl, Z is benzyloxycarbonyl, SEt is ethanethiol, and NMe_2 is dimethylamino.

10. The ^{13}C- or ^{14}C-labelled compound or salt thereof for use in diagnosis according to claim 9, wherein

1) in the case where X_2 is a protecting group selected from the group consisting of Dansyl, Cbz, Ac and Z, and R_2 is an amino acid or a peptide of 2 amino acids,

1-i) when Z_1 is D-Ala, Y_2 is an amino acid other than Val,
1-ii) when Z_1 is Ala, Y_2 is an amino acid other than Leu,
1-iii) when Z_1 is Gly-OEt, Y_2 is an amino acid other than Pro,
1-iv) when Z_1 is Gln(NMe_2) or Gln(NMe_2)-SEt, Y_2 is an amino acid other than Ala,

2) in the case where X_2 is Ac or Boc, and R_2 is a single bond,

2-i) when Z_1 is D-Ala, Y_2 is an amino acid other than D-Ala,
2-ii) when Z_1 is Pro-OMe, Y_2 is an amino acid other than Gly,
2-iii) when Z_1 is Ala-OMe, Y_2 is an amino acid other than Leu,

3) in the case where X_2 is a hydrogen atom and R_2 is an amino acid or a peptide of 2 or 3 amino acids,

3-i) when Z_1 is Leu, Y_2 is an amino acid other than Pro, Leu and Phe,
3-ii) when Z_1 is Phe, Y_2 is an amino acid other than Pro,
3-iii) when Z_1 is Gly, Y_2 is an amino acid other than Gly,
3-iv) when Z_1 is Pro, Y_2 is an amino acid other than Leu,
3-v) when Z_1 is Met, Y_2 is an amino acid other than Asp and Phe,
3-vi) when Z_1 is Asn-Bzl, Y_2 is an amino acid other than Leu,
3-vii) when Z_1 is Ser, Y_2 is an amino acid other than Asp, and

4) in the case where X_2 is a hydrogen atom and R_2 is a single bond,

4-i) when Z_1 is Pro,Y_2 is an amino acid other than Ala,
4-ii) when Z_1 is Leu , Y_2 is an amino acid other than Gly,Val,Leu and Tyr,
4-iii) when Z_1 is Phe, Y_2 is an amino acid other than Gly,
4-iv) when Z_1 is Gly-OEt, Y_2 is an amino acid other than Gly,
4-v) when Z_1 is Ala-OMe, Y_2 is an amino acid other than Leu.

11. The ^{13}C- or ^{14}C-labelled compound or salt thereof for use in diagnosis according to claim 9 or 10, wherein X_2 is selected from the group consisting of Ac, Bz (benzoyl), Boc, Z and a hydrogen atom.

12. The ^{13}C- or ^{14}C-labelled compound or salt thereof for use in diagnosis according to any one of claims 9 to 11, wherein the compound, or salt thereof is

capable of being a substrate of a protease or proteases such that it is subsequently capable of being decarboxylated to generate $^{13}CO_2$ or $^{14}CO_2$.

13. The ^{13}C- or ^{14}C-labelled compound or salt thereof for use in diagnosis according to claim 12, wherein the protease or proteases are pancreatic exocrine proteases.

14. The ^{13}C- or ^{14}C-labelled compound or salt thereof for use in diagnosis according to claim 13, wherein the pancreatic exocrine protease or proteases are selected from the group consisting of chymotrypsin, trypsin, elastase, and carboxypeptidases.

15. The ^{13}C- or ^{14}C-labelled compound or salt thereof for use in diagnosis according to any one of claims 9 to 14, wherein X_2 is a protecting group and R_2 is a single bond.

16. The ^{13}C- or ^{14}C-labelled compound or salt thereof for use in diagnosis according to any one of claims 9 to 15, wherein

(1) at least one of the amino acids in Y_2 and Z_1 is Arg or Lys,
(2) at least one of the amino acids in Y_2 and Z_1 is an aromatic amino acid, Leu, His or Met,
(3) at least one of the amino acids in Y_2 and Z_1 is a neutral and non-aromatic amino acid,
(4) the amino acid in Z_1 is an amino acid other than Arg, Lys and Pro, or
(5) the amino acid in Z_1 is Arg or Lys.

17. The ^{13}C- or ^{14}C-labelled compound or salt thereof for use in diagnosis according to any one of claims 9 to 16, wherein X_2 is selected from the group consisting of a hydrogen atom, Bz, Ac and Boc,Y_2 is selected from the group consisting of Phe, Ala, Gly, Tyr and Arg, Z_1 is selected from the group consisting of Leu optionally having a protecting group, Ala optionally having a protecting group, and Gly optionally having a protecting group.

18. The ^{13}C- or ^{14}C-labelled compound or salt thereof for use in diagnosis according to claim 17, wherein Z_1 is selected from the group consisting of Leu, Ala, Gly, Leu-OMe, Leu-OEt, Ala-OMe, Ala-OEt, Gly-OMe and Gly-OEt.

19. The ^{13}C- or ^{14}C-labelled compound or salt thereof for use in diagnosis according to any one of claims 9 to 18, wherein Z_1 is a ^{13}C- or ^{14}C-labelled amino acid optionally having a protecting group.

20. The ^{13}C- or ^{14}C-labelled compound or salt thereof for use in diagnosis according to claim 9, which is selected from the group consisting of the following compounds:

(a) Phe-^{13}C-Leu,
(b) Arg-^{13}C-Leu,
(c) Bz-Ala-^{13}C-ALa,
(d) Bz-Gly-^{13}C-Leu,
(e) Bz-Phe-^{13}C-Gly,
(f) Bz-Tyr-^{13}C-Leu,
(g) Bz-Phe-^{13}C-Leu,
(h) Bz-(DL)Phe-^{13}C-Leu,
(j) Bz-Arg-^{13}C-Leu,
(k) Ac-Phe-^{13}C-Leu,
(l) Ac-Tyr-^{13}C-Leu,
(m) Bz-Ala-^{13}C-Ala-OMe,
(n) Bz-Gly-^{13}C-Leu-OMe,
(o) Bz-Phe-^{13}C-Gly-OMe,
(p) Bz-Phe-^{13}C-Leu-OMe,
(q) Bz-(DL)Phe-^{13}C-Leu-OMe,
(r) Ac-Phe-^{13}C-Leu-OMe,
(s) Ac-Tyr-^{13}C-Leu-OMe,
(t) Bz-Ala-Ala-Ala-Ala-Gly-Phe-^{13}C-Leu,
(u) Boc-Ala-Ala-Ala-Ala-Gly-Phe-^{13}C-Leu, and
(v) Bz-Ala-Ala-Ala-Ala-^{13}C-Gly-Phe-Leu

21. Use of a ^{13}C- or ^{14}C-labelled compound as defined in any one of claims 9 to 20 or a pharmaceutically acceptable salt thereof in the manufacture of a diagnostic agent for pancreatic exocrine function, wherein the diagnostic agent is used in a breath test.

22. Use of a ^{13}C- or ^{14}C-labelled compound as defined in any one of claims 9 to 20 or a pharmaceutically acceptable salt thereof in the manufacture of a diagnostic agent for chronic pancreatitis or acute pancreatitis.

Patentansprüche

1. Verwendung einer Aminosäure oder eines Peptids, die/das wenigstens ein ^{13}C- oder ^{14}C-Atom enthält, oder ein pharmazeutisch verträgliches Salz davon, welches nicht Bz-Tyr-^{13}C-PABA ist, bei der Herstellung eines Diagnosemittels für Pankreasexokrinfunktion, wobei das Diagnosemittel in einem Atemtest verwendet wird.

2. Verwendung nach Anspruch 1, wobei das Aminosäuremolekül ein ^{13}C- oder ^{14}C-Atom enthält.

3. Verwendung nach Anspruch 1, wobei die Aminosäure oder das Peptid eine modifizierende oder schützende Gruppe aufweist und die modifizierende oder schützende Gruppe ein ^{13}C- oder ^{14}C-Atom enthält.

4. Verwendung nach einem der Ansprüche 1 bis 3, wo-

bei die Aminosäure oder das Peptid durch die folgende Formel (I) wiedergegeben wird:

$$X_1\text{-}R_1\text{-}Y_1 \qquad (I),$$

worin X_1 ein Wasserstoffatom oder eine Schutzgruppe ist,
R_1 ein Peptid mit 2 bis 50 Aminosäuren, eine Aminosäure oder eine Einfachbindung ist,
Y_1 eine Aminosäure ist, die optional eine Schutzgruppe aufweist.

5. Verwendung nach Anspruch 4, wobei wenigstens eine der Aminosäuren in R_1 und Y_1 oder wenigstens eine Estergruppe in Y_1, wenn die Aminosäure in Y_1 mit der Estergruppe geschützt ist, mit ^{13}C oder ^{14}C markiert ist.

6. Verwendung nach einem der vorangegangenen Ansprüche, wobei die Aminosäure oder das Peptid oder das pharmazeutisch verträgliche Salz davon ein Substrat für eine Protease oder für Proteasen sein kann, so daß sie/es anschließend unter Erzeugung von $^{13}CO_2$ oder $^{14}CO_2$ decarboxyliert werden kann.

7. Verwendung nach Anspruch 6, wobei die Protease oder die Proteasen Pankreasexokrinproteasen sind.

8. Verwendung nach Anspruch 7, wobei die Pankreasexokrinprotease oder die -proteasen aus der Gruppe ausgewählt ist/sind, bestehend aus Chymotrypsin, Trypsin, Elastase und Carboxypeptidasen.

9. Eine mit ^{13}C oder ^{14}C markierte Verbindung oder ein Salz davon für die Verwendung in der Diagnose, wobei die mit ^{13}C oder ^{14}C markierte Verbindung durch die folgende Formel (II) wiedergegeben wird:

$$X_2\text{-}R_2\text{-}Y_2\text{-}Z_1 \qquad (II),$$

worin X_2 ein Wasserstoffatom oder eine Schutzgruppe ist,
R_2 ein Peptid mit 2 bis 5 Aminosäuren, eine Aminosäure oder eine Einfachbindung ist,
Y_2 eine Aminosäure ist,
Z_1 eine Aminosäure ist, die optional eine Schutzgruppe aufweist, und wobei wenigstens eine der Aminosäuren in R_2, Y_2 und Z_1 oder wenigstens eine der Schutzgruppen in X_2 und Z_1, wenn die Schutzgruppen ein Kohlenstoffatom enthalten, mit ^{13}C oder ^{14}C markiert ist, und worin

1) in dem Fall, daß X_2 eine Schutzgruppe und R_2 eine Einfachbindung ist,

1-i) Y_2 eine andere Aminosäure als D-Ala ist, wenn Z_1 D-Ala ist,
1-ii) Y_2 eine andere Aminosäure als Leu ist, wenn Z_1 Ala-OMe ist,
1-iii) Y_2 eine andere Aminosäure als Gly ist, wenn Z_1 Pro-OMe ist,

2) in dem Fall, daß X_2 eine Schutzgruppe und R_2 eine Aminosäure ist,

2-i) Y_2 eine andere Aminosäure als Val ist, wenn Z_1 D-Ala ist,
2-ii) Y_2 eine andere Aminosäure als Leu ist, wenn Z_1 Ala ist,
2-iii) Y_2 eine andere Aminosäure als Pro, wenn Z_1 Gly-OEt ist,

3) in dem Fall, daß X_2 eine Schutzgruppe, R_2 ein Peptid mit zwei Aminosäuren und Z_1 Gln, welches optional SEt aufweist, ist, Y_2 eine andere Aminosäure als Ala ist,
4) in dem Fall, daß X_2 ein Wasserstoffatom und R_2 eine Einfachbindung ist,

4-i) Y_2 eine andere Aminosäure als Ala ist, wenn Z_1 Pro ist,
4-ii) Y_2 eine andere Aminosäure als Gly, Val, Leu und Tyr ist, wenn Z_1 Leu ist,
4-iii) Y_2 eine andere Aminosäure als Gly ist, wenn Z_1 Phe ist,
4-iv) Y_2 eine andere Aminosäure als Gly ist, wenn Z_1 Gly-OEt ist,
4-v) Y_2 eine andere Aminosäure als Leu ist, wenn Z_1 Ala-OMe ist,

5) in dem Fall, daß X_2 ein Wasserstoffatom und R_2 eine Aminosäure ist,

5-i) Y_2 eine andere Aminosäure als Pro ist, wenn Z_1 Leu ist,
5-ii) Y_2 eine andere Aminosäure als Pro ist, wenn Z_1 Phe ist,
5-iii) Y_2 eine andere Aminosäure als Gly ist, wenn Z_1 Gly ist,
5-iv) Y_2 eine andere Aminosäure als Leu ist, wenn Z_1 Pro ist,
5-v) Y_2 eine andere Aminosäure als Asp ist, wenn Z_1 Met ist,
5-vi) Y_2 eine andere Aminosäure als Leu ist, wenn Z_1 Asn-Bzl ist,

6) in dem Fall, daß X_2 ein Wasserstoffatom und R_2 ein Peptid aus 2 Aminosäuren ist,

6-i) Y_2 eine andere Aminosäure als Leu ist,

wenn Z_1 Leu ist,
6-ii) Y_2 eine andere Aminosäure als Asp ist, wenn Z_1 Ser ist, und

7) in dem Fall, daß X_2 ein Wasserstoffatom und R_2 ein Peptid aus 3 Aminosäuren ist,

7-i) Y_2 eine andere Aminosäure als Phe ist, wenn Z_1 Leu ist,
7-ii) Y_2 eine andere Aminosäure als Phe ist, wenn Z_1 Met ist,

unter der Bedingung, daß die folgenden Verbindungen ausgeschlossen sind:
Ala-Pro, Gly-Leu, Gly-Phe, Val-Leu, Leu-Leu, Tyr-Leu, Ac-D-Ala-D-Ala, Gly-Gly-OEt, Leu-Ala-OMe, Ac-Gly-Pro-OMe, Boc-Leu-Ala-OMe, Gly-Pro-Leu, Gly-Pro-Phe, Ala-Gly-Gly, Gly-Leu-Pro, Phe-Asp-Met, Gly-Leu-Pro, Dansyl-Tyr-Val-D-Ala, Cbz-Gly-Leu-Ala, Thr-Leu-Asn-Bzl, Z-Pro-Pro-Gly-OEt, Leu-Leu-Leu-Leu, Lys-Arg-Asp-Ser, Ac-Leu-Ala-Ala-Gln (NMe_2), Ac-Leu-Ala-Ala-Gln (NMe_2)-SEt, Tyr-Gly-Gly-Phe-Leu und Tyr-Gly-Gly-Phe-Met, worin Ac Acetyl ist, Et Ethyl ist, Me Methyl ist, Boc t-Butyloxycarbonyl ist, Dansyl Dansyl ist, Cbz Carbobenzyloxy ist, Bzl Benzoyl ist, Z Benzyloxycarbonyl ist, SEt Ethanthiol ist und NMe_2 Dimethylamino ist.

10. Mit ^{13}C oder ^{14}C markierte Verbindung oder Salz davon für die Verwendung in der Diagnose gemäß Anspruch 9, worin

1) in dem Fall, daß X_2 eine Schutzgruppe ist, die aus der Gruppe ausgewählt ist, bestehend aus Dansyl, Cbz, Ac und Z, und R_2 eine Aminosäure oder ein Peptid mit 2 Aminosäuren ist,

1-i) Y_2 eine andere Aminosäure als Val ist, wenn Z_1 D-Ala ist,
1-ii) Y_2 eine andere Aminosäure als Leu ist, wenn Z_1 Ala ist,
1-iii) Y_2 eine andere Aminosäure als Pro ist, wenn Z_1 Gly-OEt ist,
1-iv) Y_2 eine andere Aminosäure als Ala ist, wenn Z_1 Gln(NMe_2) oder Gln(NMe_2) -SEt ist,

2) in dem Fall, daß X_2 Ac oder Boc und R_2 eine Einfachbindung ist,

2-i) Y_2 eine andere Aminosäure als D-Ala ist, wenn Z_1 D-Ala ist,
2-ii) Y_2 eine andere Aminosäure als Gly ist, wenn Z_1 Pro-OMe ist,
2-iii) Y_2 eine andere Aminosäure als Leu ist, wenn Z_1 Ala-OMe ist,

3) in dem Fall, daß X_2 ein Wasserstoffatom und R_2 eine Aminosäure oder ein Peptid aus 2 oder 3 Aminosäuren ist,

3-i) Y_2 eine andere Aminosäure als Pro, Leu und Phe ist, wenn Z_1 Leu ist,
3-ii) Y_2 eine andere Aminosäure als Pro ist, wenn Z_1 Phe ist,
3-iii) Y_2 eine andere Aminosäure als Gly ist, wenn Z_1 Gly ist,
3-iv) Y_2 eine andere Aminosäure als Leu ist, wenn Z_1 Pro ist,
3-v) Y_2 eine andere Aminosäure als Asp und Phe ist, wenn Z_1 Met ist,
3-vi) Y_2 eine andere Aminosäure als Leu ist, wenn Z_1 Asn-Bzl ist,
3-vii) Y_2 eine andere Aminosäure als Asp ist, wenn Z_1 Ser ist,

4) in dem Fall, daß X_2 ein Wasserstoffatom und R_2 eine Einfachbindung ist,

4-i) Y_2 eine andere Aminosäure als Ala ist, wenn Z_1 Pro ist,
4-ii) Y_2 eine andere Aminosäure als Gly, Val, Leu und Tyr ist, wenn Z_1 Leu ist,
4-iii) Y_2 eine andere Aminosäure als Gly ist, wenn Z_1 Phe ist,
4-iv) Y_2 eine andere Aminosäure als Gly ist, wenn Z_1 Gly-OEt ist,
4-v) Y_2 eine andere Aminosäure als Leu ist, wenn Z_1 Ala-OMe ist.

11. Mit ^{13}C oder ^{14}C markierte Verbindung oder Salz davon für die Verwendung in der Diagnose gemäß Anspruch 9 oder 10, worin X_2 aus der Gruppe ausgewählt ist, bestehend aus Ac, Bz (Benzoyl), Boc, Z und einem Wasserstoffatom.

12. Mit ^{13}C oder ^{14}C markierte Verbindung oder Salz davon für die Verwendung in der Diagnose nach einem der Ansprüche 9 bis 11, wobei die Verbindung oder das Salz davon ein Substrat für eine Protease oder für Proteasen sein kann, so daß sie/es anschließend unter Erzeugung von $^{13}CO_2$ oder $^{14}CO_2$ decarboxyliert werden kann.

13. Mit ^{13}C oder ^{14}C markierte Verbindung oder Salz davon für die Verwendung in der Diagnose gemäß Anspruch 12, wobei die Protease oder die Proteasen Pankreasexokrinproteasen sind.

14. Mit ^{13}C oder ^{14}C markierte Verbindung oder Salz davon für die Verwendung in der Diagnose gemäß Anspruch 13, wobei die Pankreasexokrinprotease oder die -proteasen aus der Gruppe ausgewählt sind, bestehend aus Chymotrypsin, Trypsin, Elastase und Carboxypeptidasen.

15. Mit ^{13}C oder ^{14}C markierte Verbindung oder Salz davon für die Verwendung in der Diagnose gemäß einem der Ansprüche 9 bis 14, wobei X_2 eine Schutzgruppe und R_2 eine Einfachbindung ist.

16. Mit ^{13}C oder ^{14}C markierte Verbindung oder Salz davon für die Verwendung in der Diagnose gemäß einem der Ansprüche 9 bis 15, worin

(1) wenigstens eine der Aminosäuren in Y_2 und Z_1 Arg oder Lys ist,
(2) wenigstens eine der Aminosäuren in Y_2 und Z_1 eine aromatische Aminosäure, Leu, His oder Met ist,
(3) wenigstens eine der Aminosäuren in Y_2 und Z_1 eine neutrale und nicht-aromatische Aminosäure ist,
(4) die Aminosäure in Z_1 eine andere Aminosäure als Arg, Lys und Pro ist oder
(5) die Aminosäure in Z_1 Arg oder Lys ist.

17. Mit ^{13}C oder ^{14}C markierte Verbindung oder Salz davon für die Verwendung in der Diagnose gemäß einem der Ansprüche 9 bis 16, worin X_2 aus der Gruppe ausgewählt ist, bestehend aus einem Wasserstoffatom, Bz, Ac und Boc, Y_2 aus der Gruppe ausgewählt ist, bestehend aus Phe, Ala, Gly, Tyr und Arg, Z_1 aus der Gruppe ausgewählt ist, bestehend aus Leu, welches optional eine Schutzgruppe aufweist, Ala, welches optional eine Schutzgruppe aufweist, und Gly, welches optional eine Schutzgruppe aufweist.

18. Mit ^{13}C oder ^{14}C markierte Verbindung oder Salz davon für die Verwendung in der Diagnose gemäß Anspruch 17, worin Z_1 aus der Gruppe ausgewählt ist, bestehend aus Leu, Ala, Gly, Leu-OMe, Leu-OEt, Ala-OMe, Ala-OEt, Gly-OMe und Gly-OEt.

19. Mit ^{13}C oder ^{14}C markierte Verbindung oder Salz davon für die Verwendung in der Diagnose gemäß einem der Ansprüche 9 bis 18, worin Z_1 eine mit ^{13}C oder ^{14}C markierte Aminosäure ist, die optional eine Schutzgruppe aufweist.

20. Mit ^{13}C oder ^{14}C markierte Verbindung oder Salz davon für die Verwendung in der Diagnose gemäß Anspruch 9, welche aus der Gruppe ausgewählt ist, die aus den folgenden Verbindungen besteht:

(a) Phe-^{13}C-Leu,
(b) Arg-^{13}C-Leu,
(c) Bz-Ala-^{13}C-Ala,
(d) Bz-Gly-^{13}C-Leu,
(e) Bz-Phe-^{13}C-Gly,
(f) Bz-Tyr-^{13}C-Leu,
(g) Bz-Phe-^{13}C-Leu,
(h) Bz-(DL)Phe-^{13}C-Leu,
(j) Bz-Arg-^{13}C-Leu,
(k) Ac-Phe-^{13}C-Leu,
(l) Ac-Tyr-^{13}C-Leu,
(m) Bz-Ala-^{13}C-Ala-OMe,
(n) Bz-Gly-^{13}C-Leu-OMe,
(o) Bz-Phe-^{13}C-Gly-OMe,
(p) Bz-Phe-^{13}C-Leu-OMe,
(q) Bz-(DL)Phe-^{13}C-Leu-OMe,
(r) Ac-Phe-^{13}C-Leu-OMe,
(s) Ac-Tyr-^{13}C-Leu-OMe,
(t) Bz-Ala-Ala-Ala-Ala-Gly-Phe-^{13}C-Leu,
(u) Boc-Ala-Ala-Ala-Ala-Gly-Phe-^{13}C-Leu und
(v) Bz-Ala-Ala-Ala-Ala-^{13}C-Gly-Phe-Leu.

21. Verwendung einer mit ^{13}C oder ^{14}C markierten Verbindung nach einem der Ansprüche 9 bis 20 oder eines pharmazeutisch verträglichen Salzes davon bei der Herstellung eines Diagnosemittels für Pankreasexokrinfunktion, wobei das Diagnosemittel in einem Atemtest verwendet wird.

22. Verwendung einer mit ^{13}C oder ^{14}C markierten Verbindung nach einem der Ansprüche 9 bis 20 oder eines pharmazeutisch verträglichen Salzes davon bei der Herstellung eines Diagnosemittels für chronische Pankreatitis oder akute Pankreatitis.

Revendications

1. Utilisation d'un aminoacide ou d'un peptide contenant au moins un atome de ^{13}C ou ^{14}C, ou d'un de ses sels pharmaceutiquement acceptables autre que Bz-Tyr-^{13}C-PABA dans la production d'un agent de diagnostic pour la fonction exocrine pancréatique, dans laquelle l'agent de diagnostic est utilisé dans un test respiratoire.

2. Utilisation suivant la revendication 1, dans laquelle la molécule d'aminoacide contient un atome de ^{13}C ou ^{14}C.

3. Utilisation suivant la revendication 1, dans laquelle l'aminoacide ou le peptide possède un groupe modificateur ou protecteur et le groupe modificateur ou protecteur contient un atome de ^{13}C ou ^{14}C.

4. Utilisation suivant l'une quelconque des revendications 1 à 3, dans laquelle l'aminoacide ou le peptide est représenté par la formule (I) suivante :

$$X_1\text{-}R_1\text{-}Y_1 \qquad (I)$$

dans laquelle X_1 représente un atome d'hydrogène ou un groupe protecteur,

R_1 représente un peptide de 2 à 50 aminoacides, un aminoacide ou une liaison simple,
Y_1 représente un aminoacide comprenant facultativement un groupe protecteur.

5. Utilisation suivant la revendication 4, dans laquelle au moins un des aminoacides dans R_1 et Y_1, ou au moins un groupe ester dans Y_1 lorsque l'aminoacide dans Y_1 est protégé avec le groupe ester, est marqué avec ^{13}C ou ^{14}C.

6. Utilisation suivant l'une quelconque des revendications précédentes, dans laquelle l'aminoacide ou le peptide, ou un de ses sels pharmaceutiquement acceptables, est capable d'être un substrat d'une ou plusieurs protéase de telle sorte qu'il puisse ensuite être décarboxylé pour engendrer du $^{13}CO_2$ ou du $^{14}CO_2$.

7. Utilisation suivant la revendication 6, dans laquelle la ou les protéases sont des protéases exocrines pancréatiques.

8. Utilisation suivant la revendication 7, dans laquelle la ou les protéases exocrines pancréatiques sont choisies dans le groupe consistant en la chymotrypsine, la trypsine, l'élastase et des carboxypeptidases.

9. Composé ou un de ses sels marqué avec ^{13}C ou ^{14}C, destiné à être utilisé en diagnostic, ledit composé marqué avec ^{13}C ou ^{14}C étant représenté par la formule (II) suivante :

$$X_2\text{-}R_2\text{-}Y_2\text{-}Z_1 \qquad \text{(II)}$$

dans laquelle X_2 représente un atome d'hydrogène ou un groupe protecteur,
R_2 représente un peptide de 2 à 5 aminoacides, un aminoacide ou une liaison simple,
Y_2 représente un aminoacide,
Z_1 représente un aminoacide comprenant facultativement un groupe protecteur, et au moins un des aminoacides dans R_2, Y_2 et Z_1, ou au moins un des groupes protecteurs de X_2 et Z_1 lorsque les groupes protecteurs contiennent un atome de carbone, est marqué avec ^{13}C ou ^{14}C,
et dans laquelle

1) dans le cas où X_2 représente un groupe protecteur et R_2 représente une liaison simple,

1-i) lorsque Z_1 représente un groupe D-Ala, Y_2 représente un aminoacide autre que D-Ala,
1-ii) lorsque Z_1 représente Ala-OMe, Y_2 représente un aminoacide autre que Leu,
1-iii) lorsque Z_1 représente Pro-OMe, Y_2 représente un aminoacide autre que Gly,

2) dans le cas où X_2 représente un groupe protecteur et R_2 représente un aminoacide,

2-i) lorsque Z_1 représente D-Ala, Y_2 représente un aminoacide autre que Val,
2-ii) lorsque Z_1 représente Ala, Y_2 représente un aminoacide autre que Leu,
2-iii) lorsque Z_1 représente Gly-OEt, Y_2 représente un aminoacide autre que Pro,

3) dans le cas où X_2 représente un groupe protecteur, R_2 représente un peptide de 2 aminoacides et Z_1 représente Gln comprenant facultativement SEt, Y_2 représente un aminoacide autre que Ala,
4) dans le cas où X_2 représente un atome d'hydrogène et R_2 représente une liaison simple,

4-i) lorsque Z_1 représente Pro, Y_2 représente un aminoacide autre que Ala,
4-ii) lorsque Z_1 représente Leu, Y_2 représente un aminoacide autre Gly, Val, Leu et Tyr,
4-iii) lorsque Z_1 représente Phe, Y_2 représente un aminoacide autre que Gly,
4-iv) lorsque Z_1 représente Gly-Oet, Y_2 représente un aminoacide autre que Gly,
4-v) lorsque Z_1 représente Ala-OMe, Y_2 représente un aminoacide autre que Leu,

5) dans le cas où X_2 représente un atome d'hydrogène et R_2 représente un aminoacide,

5-i) lorsque Z_1 représente Leu, Y_2 représente un aminoacide autre que Pro,
5-ii) lorsque Z_1 représente Phe, Y_2 représente un aminoacide autre que Pro,
5-iii) lorsque Z_1 représente Gly, Y_2 représente un aminoacide autre que Gly,
5-iv) lorsque Z_1 représente Pro, Y_2 représente un aminoacide autre que Leu,
5-v) lorsque Z_1 représente Met, Y_2 représente un aminoacide autre que Asp,
5-vi) lorsque Z_1 représente Asn-Bzl, Y_2 représente un aminoacide autre que Leu,

6) dans le cas où X_2 représente un atome d'hydrogène et R_2 représente un peptide de 2 aminoacides,

6-i) lorsque Z_1 représente Leu, Y_2 représente un aminoacide autre que Leu,
6-ii) lorsque Z_1 représente Ser, Y_2 représente un aminoacide autre que Asp, et

7) dans le cas où X_2 représente un atome d'hydrogène et R_2 représente un peptide de 3 aminoacides,

7-i) lorsque Z_1 représente Leu, Y_2 représente un aminoacide autre que Phe,
7-ii) lorsque Z_1 représente Met, Y_2 représente un aminoacide autre que Phe,

sous réserve que les composés suivants soient exclus :
Ala-Pro, Gly-Leu, Gly-Phe, Val-Leu, Leu-Leu, Tyr-Leu, Ac-D-Ala-D-Ala, Gly-Gly-OEt, Leu-Ala-OMe, Ac-Gly-Pro-OMe, Boc-Leu-Ala-OMe, Gly-Pro-Leu, Gly-Pro-Phe, Ala-Gly-Gly, Gly-Leu-Pro, Phe-Asp-Met, Gly-Leu-Pro, Dansyl-Tyr-Val-D-Ala, Cbz-Gly-Leu-Ala, Thr-Leu-Asn-Bzl, Z-Pro-Pro-Gly-OEt, Leu-Leu-Leu-Leu, Lys-Arg-Asp-Ser, Ac-Leu-Ala-Gin (NMe_2), Ac-Leu-Ala-Ala-Gin (NMe_2)-SEt, Tyr-Gly-Gly-Phe-Leu et Tyr-Gly-Gly-Phe-Met, dans lesquels Ac représente un groupe acétyle, Et représente un groupe éthyle, Me représente un groupe méthyle, Boc représente un groupe tertio-butyloxycarbonyle, Dansyl désigne le groupe dansyle, Cbz représente un groupe carbobenzyloxy, Bzl représente un groupe benzoyle, Z représente un groupe benzyloxycarbonyle, SEt représente un groupe éthanethiol, et NMe_2 représente un groupe diméthylamino.

10. Composé ou un de ses sels marqué avec ^{13}C ou ^{14}C, destiné à être utilisé en diagnostic, suivant la revendication 9, dans lequel

1) dans le cas où X_2 représente un groupe protecteur choisi dans le groupe consistant en Dansyl, Cbz, Ac et Z et R_2 représente un aminoacide ou un peptide de 2 aminoacides,

1-i) lorsque Z_1 représente D-Ala, Y_2 représente un aminoacide autre que Val,
1-ii) lorsque Z_1 représente Ala, Y_2 représente un aminoacide autre Leu,
1-iii) lorsque Z_1 représente Gly-OEt, Y_2 représente un aminoacide autre que Pro,
1-iv) lorsque Z_1 représente Gln(NMe_2) ou Gln(NMe_2)-SEt, Y_2 représente un aminoacide autre que Ala,

2) dans le cas où X_2 représente Ac ou Boc, et R2 représente une liaison simple,

2-i) lorsque Z1 représente D-Ala, Y_2 représente un aminoacide autre que D-Ala,
2-ii) lorsque Z_1 représente Pro-OMe, Y_2 représente un aminoacide autre que Gly,
2-iii) lorsque Z_1 représente Ala-OMe, Y_2 représente un aminoacide autre que Leu,

3) dans le cas où X_2 représente un atome d'hydrogène et R_2 représente un aminoacide ou un peptide de 2 ou 3 aminoacides

3-i) lorsque Z_1 représente Leu, Y_2 représente un aminoacide autre que Pro, Leu et Phe,
3-ii) lorsque Z_1 représente Phe, Y_2 représente un aminoacide autre que Pro,
3-iii) lorsque Z_1 représente Gly, Y_2 représente un aminoacide autre que Gly,
3-iv) lorsque Z_1 représente Pro, Y_2 représente un aminoacide autre que Leu,
3-v) lorsque Z_1 représente Met, Y_2 représente un aminoacide autre que Asp et Phe,
3-vi) lorsque Z_1 représente Asn-Bzl, Y_2 représente un aminoacide autre Leu,
3-vii) lorsque Z_1 représente Ser, Y_2 représente un aminoacide autre que Asp, et

4) dans le cas où X_2 représente un atome d'hydrogène et R_2 représente une liaison simple,

4-i) lorsque Z_1 représente Pro, Y_2 représente un aminoacide autre que Ala,
4-ii) lorsque Z_1 représente Leu, Y_2 représente un aminoacide autre que Gly, Val, Leu et Tyr,
4-iii) lorsque Z_1 représente Phe, Y_2 représente un aminoacide autre que Gly,
4-iv) lorsque Z_1 représente Gly-OEt, Y_2 représente un aminoacide autre que Gly,
4-v) lorsque Z_1 représente Ala-OMe, Y_2 représente un aminoacide autre que Leu.

11. Composé ou un de ses sels marqué avec ^{13}C ou ^{14}C, destiné à être utilisé en diagnostic, suivant la revendication 9 ou 10, dans lequel X_2 est choisi dans le groupe consistant en Ac, Bz (benzoyle), Boc, Z et un atome d'hydrogène.

12. Composé ou un de ses sels marqué avec ^{13}C ou ^{14}C, destiné à être utilisé en diagnostic, suivant l'une quelconque des revendications 9 à 11, le composé ou son sel étant capable d'être un substrat d'une ou plusieurs protéases de telle sorte qu'il puisse ensuite être décarboxylé pour engendrer du $^{13}CO_2$ ou $^{14}CO_2$.

13. Composé ou un de ses sels marqué avec ^{13}C ou ^{14}C, destiné à être utilisé en diagnostic, suivant la revendication 12, dans lequel la ou les protéases sont des protéases exocrines pancréatiques.

14. Composé ou un de ses sels marqué avec ^{13}C ou ^{14}C, destiné à être utilisé en diagnostic, suivant la revendication 13, dans lequel la ou les protéases exocrines pancréatiques sont choisies dans le

groupe consistant en chymotrypsine, trypsine, élastase et carboxypeptidases.

15. Composé ou un de ses sels marqué avec ^{13}C ou ^{14}C, destiné à être utilisé en diagnostic, suivant l'une quelconque des revendications 9 à 14, dans lequel X_2 représente un groupe protecteur et R_2 représente une liaison simple.

16. Composé ou un de ses sels marqué avec ^{13}C ou ^{14}C, destiné à être utilisé en diagnostic, suivant l'une quelconque des revendications 9 à 15, dans lequel :

(1) au moins un des aminoacides dans Y_2 et Z_1 est Arg ou Lys,
(2) au moins un des aminoacides dans Y_2 et Z_1 est un aminoacide aromatique, Leu, His ou Met,
(3) au moins un des aminoacides dans Y_2 et Z_1 est un aminoacide neutre et non aromatique,
(4) l'aminoacide dans Z_1 est un aminoacide autre que Arg, Lys et Pro, ou
(5) l'aminoacide dans Z_1 est Arg ou Lys.

17. Composé ou un de ses sels marqué avec ^{13}C ou ^{14}C, destiné à être utilisé en diagnostic, suivant l'une quelconque des revendications 9 à 16, dans lequel X_2 est choisi dans le groupe consistant en un atome d'hydrogène, Bz, Ac et Boc, Y_2 est choisi dans le groupe consistant en Phe, Ala, Gly, Tyr et Arg, Z_1 est choisi dans le groupe consistant en Leu comprenant facultativement un groupe protecteur, Ala comprenant facultativement un groupe protecteur et Gly comprenant facultativement un groupe protecteur.

18. Composé ou un de ses sels marqué avec ^{13}C ou ^{14}C, destiné à être utilisé en diagnostic, suivant la revendication 17, dans lequel Z_1 est choisi dans le groupe consistant en Leu, Ala, Gly, Leu-OMe, Leu-OEt, Ala-OMe, Ala-OEt, Gly-OMe et Gly-OEt.

19. Composé ou un de ses sels marqué avec ^{13}C ou ^{14}C, destiné à être utilisé en diagnostic, suivant l'une quelconque des revendications 9 à 18, dans lequel Z_1 représente un aminoacide marqué avec ^{13}C ou ^{14}C comprenant facultativement un groupe protecteur.

20. Composé ou un de ses sels marqué avec ^{13}C ou ^{14}C, destiné à être utilisé en diagnostic, suivant la revendication 9, qui est choisi dans le groupe consistant en les composés suivants :

(a) Phe-^{13}C-Leu,
(b) Arg-^{13}C-Leu,
(c) Bz-Ala-^{13}C-ALa,
(d) Bz-Gly-^{13}C-Leu,
(e) Bz-Phe-^{13}C-Gly,
(f) Bz-Tyr-^{13}C-Leu,
(g) Bz-Phe-^{13}C-Leu,
(h) Bz-(DL)Phe-^{13}C-Leu,
(j) Bz-Arg-^{13}C-Leu,
(k) Ac-Phe-^{13}C-Leu,
(l) Ac-Tyr-^{13}C-Leu,
(m) Bz-Ala-^{13}C-Ala-OMe,
(n) Bz-Gly-^{13}C-Leu-OMe,
(o) Bz-Phe-^{13}C-Gly-OMe,
(p) Bz-Phe-^{13}C-Leu-Ome,
(q) Bz-(DL)Phe-^{13}C-Leu-OMe,
(r) Ac-Phe-^{13}C-Leu-OMe,
(s) Ac-Tyr-^{13}C-Leu-OMe,
(t) Bz-Ala-Ala-Ala-Ala-Gly-Phe-^{13}C-Leu,
(u) Boc-Ala-Ala-Ala-Ala-Gly-Phe-^{13}C-Leu, et
(v) Bz-Ala-Ala-Ala-Ala-^{13}C-Gly-Phe-Leu

21. Utilisation d'un composé marqué avec ^{13}C ou ^{14}C, tel que défini dans l'une quelconque des revendications 9 à 20 ou d'un de ses sels pharmaceutiquement acceptables, dans la production d'un agent de diagnostic pour la fonction exocrine pancréatique, ledit agent de diagnostic étant utilisé dans un test respiratoire.

22. Utilisation d'un composé marqué avec ^{13}C ou ^{14}C, tel que défini dans l'une quelconque des revendications 9 à 20 ou d'un de ses sels pharmaceutiquement acceptables dans la production d'un agent de diagnostic pour la pancréatite chronique ou la pancréatite aiguë.

FIG. 1

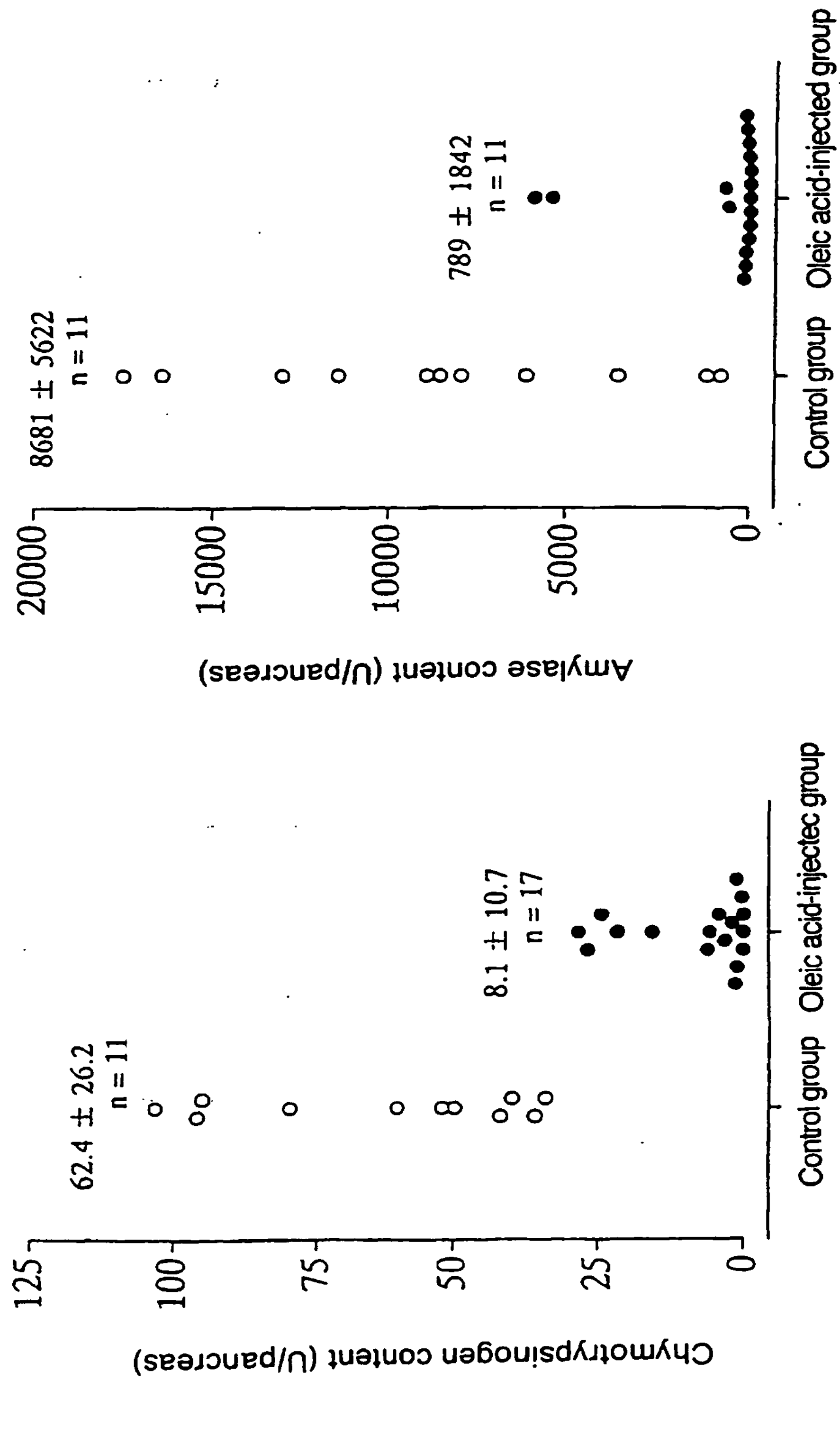

Chymotrypsinogen content (U/pancreas)
125
100
75
50
25
0
62.4 ± 26.2
n = 11
8.1 ± 10.7
n = 17
Control group
Oleic acid-injectec group
Amylase content (U/pancreas)
20000
15000
10000
5000
0
8681 ± 5622
n = 11
789 ± 1842
n = 11
Control group
Oleic acid-injected group

FIG. 2

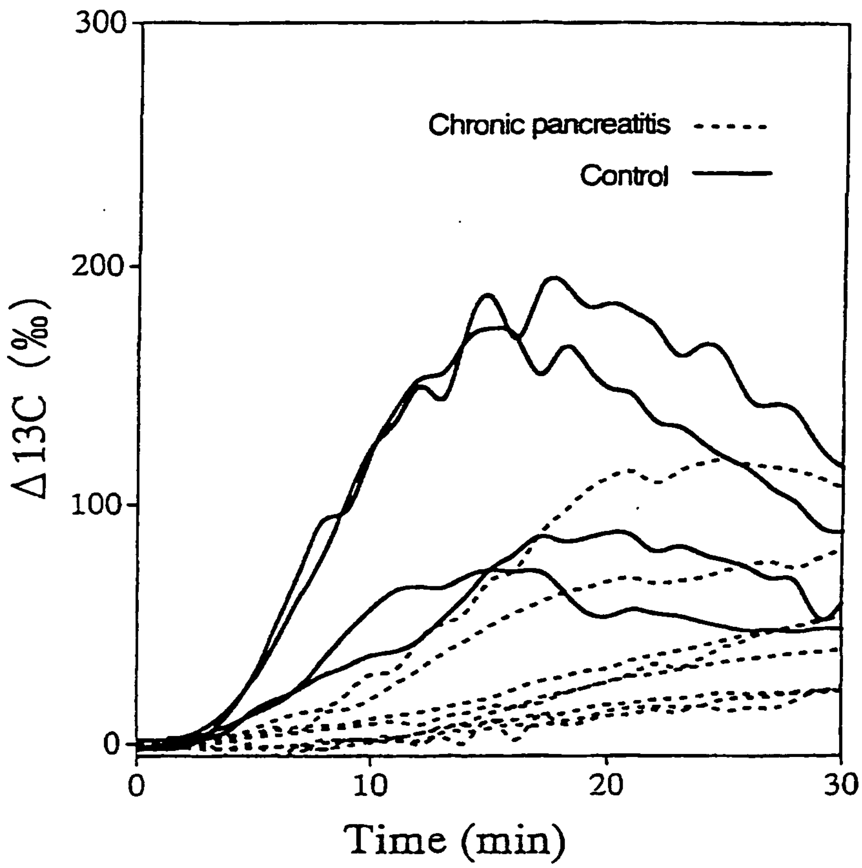

300
200
100
0
0
10
20
30
Δ 13C (‰)
Time (min)
Chronic pancreatitis
Control

FIG. 3

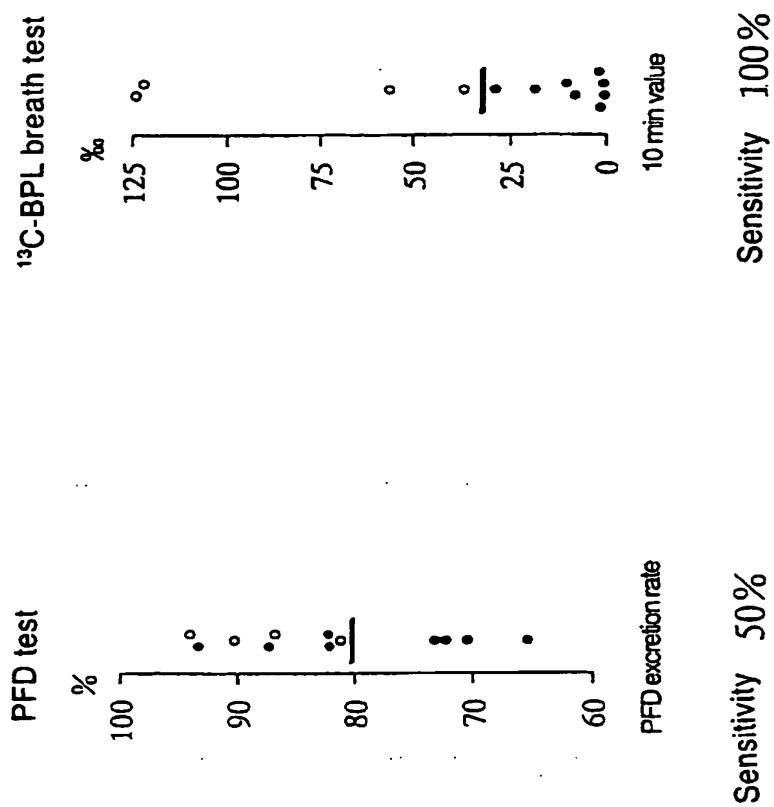

PFD test
%
100
90
80
70
60
PFD excretion rate
Sensitivity 50%
13C-BPL breath test
‰
125
100
75
50
25
0
10 min value
Sensitivity 100%

FIG. 4

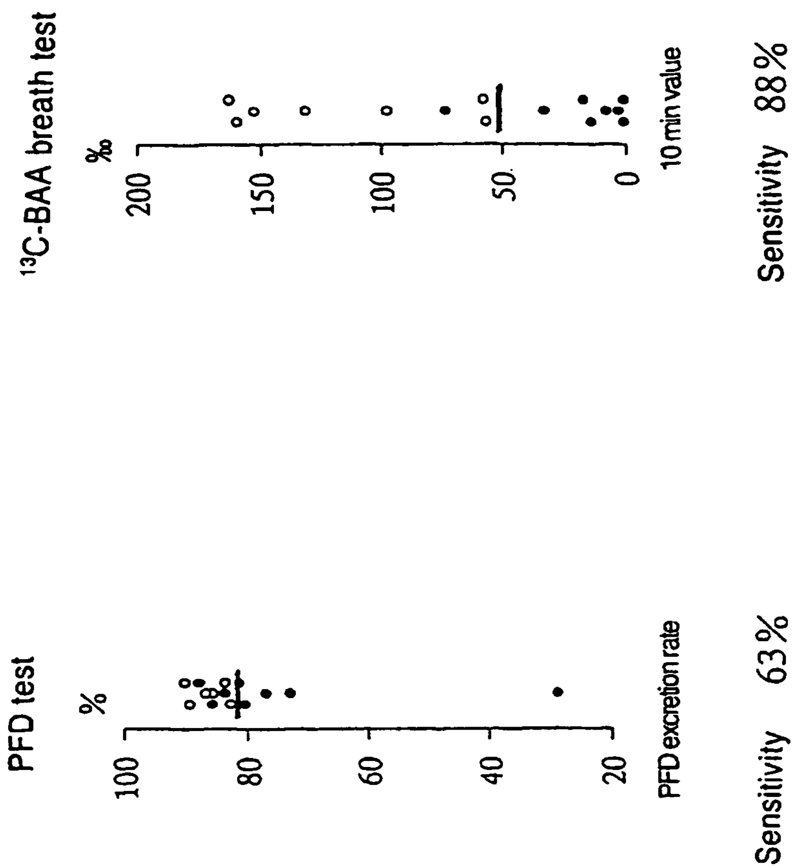
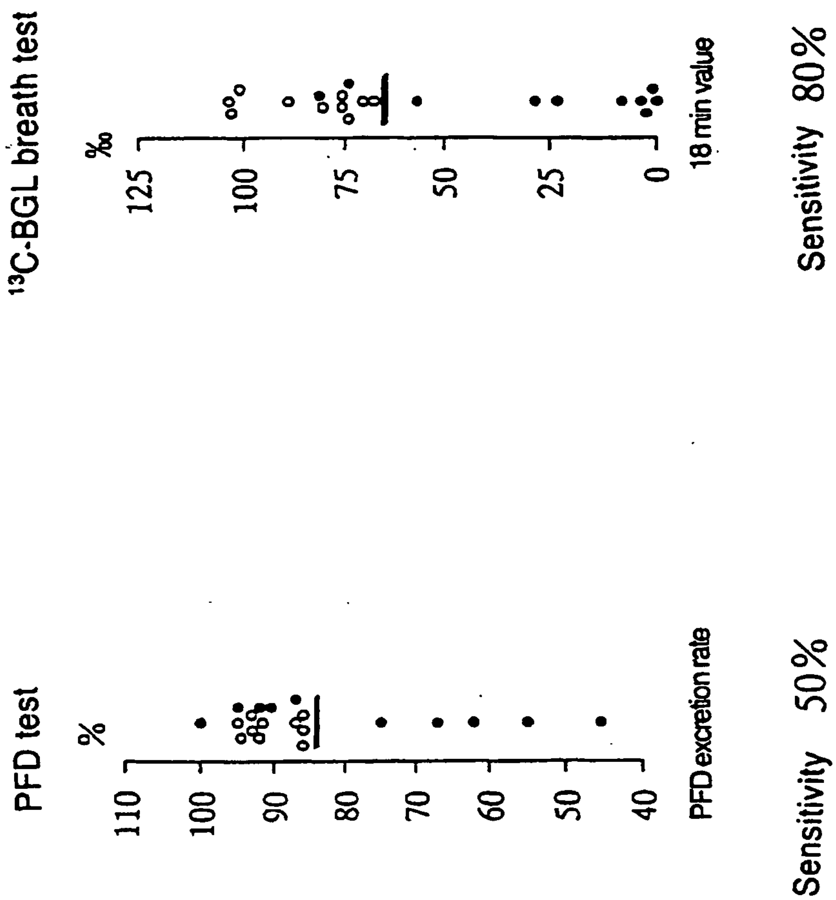

PFD test
%
100
80
60
40
20
PFD excretion rate
Sensitivity 63%
^{13}C-BAA breath test
‰
200
150
100
50
0
10 min value
Sensitivity 88%

FIG. 5


PFD test
%
110
100
90
80
70
60
50
40
PFD excretion rate
Sensitivity 50%
^{13}C-BGL breath test
‰
125
100
75
50
25
0
18 min value
Sensitivity 80%